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(54) Title: A PHARMACEUTICAL COMPOSITION FOR ADMINISTRATION OF AN ACTIVE SUBSTANCE TO OR THROUGH A SKIN OR MUCOSAL SURFACE

(57) Abstract

Pharmaceutical compositions for administration of an active substance to or through a damaged or undamaged skin or mucosal surface or to the oral cavity including the teeth of an animal such as a human. The composition has advantageous properties with respect to release of the active substance from the composition and, furthermore, the composition is bioadhesive. The composition comprises the active substance and an effective amount of a fatty acid ester which, together with a liquid phase, is capable of generating a liquid crystalline phase in which the constituents of the composition are enclosed, the active substance having a solubility in the liquid crystalline phase of at most 20 mg/g at 20 °C, and a solubility in water of at most 10 mg/ml at 20 °C, the water, where applicable, being buffered to a pH substantially identical to the pH prevailing in the liquid crystalline phase (pH about 3.6-9). The composition is particularly suited for administration of substances which have a very low water solubility and which are to be supplied in an effective amount in a localized region over a period of time. Active substances of particular importance are antiherpes virus agents including antiviral drugs and prodrugs thereof, such as nucleosides, nucleoside analogues, phosphorylated nucleosides (nucleotides), nucleotide analogues and salts, complexes and prodrugs thereof; e.g. guanosine analogues, deoxyguanosine analogues, guanine, guanine analogues, thymidine analogues, uracil analogues and adenine analogues. Especially interesting antiherpes virus agents for use either alone or in combination in a composition according to the present invention are selected from acyclovir, famciclovir, desciclovir, penciclovir, zidovudin, ganciclovir, didanosin, zalcitabin, valaciclovir, sorivudine, lobucavir, brivudine, cidofovir, n-docosanol, ISIS-2922, and prodrugs and analogues thereof.

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A PHARMACEUTICAL COMPOSITION FOR ADMINISTRATION OF AN ACTIVE SUBSTANCE TO OR THROUGH A SKIN OR MUCOSAL SURFACE

The present invention relates to a pharmaceutical composition for administration of an active substance to or through a damaged or undamaged skin or mucosal surface or to the oral cavity including the teeth of an animal such as a human. The composition is particularly suited for administration of substances which have a very low water solubility and which are to be supplied in an effective amount in a localized region over a period of time.

Background of the invention

One important known example of a composition for topical administration of a substance of very low water solubility is an ointment containing the antiviral nucleoside acyclovir. This ointment is available under the registered trade mark "Zovir®" or "Zovirax®". The release rate from this composition is rather low, and various suggestions for making topical acyclovir compositions more effective appear from the patent literature, including suggestions for increasing the effect of acyclovir by means of a potentiator or enhancer.

Disclosure of the invention

For many purposes for which acyclovir ointments are used, it would be desirable to have an ointment which could release the nucleoside at a relatively high release rate for a sufficiently long period of time in the region. An important advantage which would be obtained in this manner would be that the number of daily applications of the ointment could be reduced, such as from the present about five applications to two or three applications.

It has now been found that a particular class of systems, notably the so-called liquid crystalline phases, is capable of effectively releasing drug substances of a very low solubility. This finding must be characterized as surprising because, as it appears from the explanation which follows, the active substance in question is one having a very low solubility both in water and in the liquid crystalline phase of the composition. Furthermore, the liquid crystalline phase can confer "bioadhesion" to the composition, which means that the composition will be able to be retained for a prolonged period of time at its site of application, e.g. skin or mucosa. Thus, with such systems, it becomes realistic to considerably reduce the number of applications compared to known comp sitions.

Thus, the invention r lates to a pharmaceutical composition for administration of an active substanc to or through a damaged or undamaged skin or mucosal surface of an animal such as

a human, the composition comprising the active substance and an effective amount of a fatty acid ester which, together with a liquid phase, is capable of generating a liquid crystalline phase in which the constituents of the composition are enclosed,

the composition either being one in which the liquid crystalline phase has been generated by the fatty acid ester together with a sufficient amount of a liquid phase originally present in the composition, or the composition being in a precursor form in which fatty acid ester has not generated the liquid crystalline phase, but is capable of forming the liquid crystalline phase in situ with moisture from the surface on which the composition is applied, the moisture in this case constituting at least part of the liquid phase

the active substance having

i) a solubility in the liquid crystalline phase of at the most 20 mg/g at 20°C, and

ii) a solubility in water of at the most 10 mg/ml at 20°C, the water, where applicable, being buffered to a pH substantially identical to the pH prevailing in the liquid crystalline phase, determined as described herein, or

iii) a minimum aqueous solubility of at the most 10 mg/ml at 20°C determined at a pH in the range of 3.6-9, determined as described herein.

International Patent Application No. PCT/DK95/00143, published on 12 October, 1995 under No. WO95/26715 and being in possession of the same assignee as the present application, discloses a composition containing 2% by weight of acyclovir and 98% by weight of a glycerylmonooleate and a composition containing 5% by weight of acyclovir and 95% by weight of a glycerylmonooleate product, wherein the glycerylmonooleate product has the composition:

Glycerylmonooleate	about 84% w/w
Glycerylmonolinoleate	about 7% w/w
Saturated monoglycerides	about 7% w/w.

Therefore, for states in which the present application is co-pending with a national phase of the above international patent application (this is expressed in the claims as "where applicable"), the following proviso applies to the scope of the present application: the composition is not one consisting of either 2% by weight of acyclovir and 98% by weight of a glycerylmonooleate or 5% by weight of acyclovir and 95% by weight of a glycerylm nooleate product, wherein the glycerylmonooleate product has the composition:

Glycerylmonooleate	80-85% w/w
Glycerylmonolinoleate	5-10% w/w
Saturated monoglycerides	6-10% w/w.

As mentioned above, the pharmaceutical compositions according to the invention are intended for application to or through undamaged or damaged skin or mucosa of an animal such as a human. The mucosa is preferably selected from oral, nasal, vaginal, rectal, aural, lung, and gastrointestinal mucosa. The skin or mucosa may also be inflamed. The composition may also be administered to body cavities such as the oral cavity or by the buccal route.

Furthermore, a pharmaceutical composition according to the invention may also be applied to a nail of an animal such as a human.

In the present context the term "active substance" is intended to mean any biologically or pharmacologically active substance or antigen-comprising material; the term includes drug substances which have utility in the treatment or prevention of diseases or disorders affecting animals or humans, or in the regulation of any animal or human physiological condition and it also includes any biologically active compound or composition which, when administered in an effective amount, has an effect on living cells or organisms.

Examples of active substances of particular importance in the present context are the so-called antiherpes virus agents which have been or are developed for the treatment of herpes virus infections [herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), varicella zoster virus (VZV), cytomegalovirus (CMV), Epstein-Barr virus (EBV)]. The antiherpes virus agents include antiviral drugs and prodrugs thereof, such as nucleosides, nucleoside analogues, phosphorylated nucleosides (nucleotides), nucleotide analogues and salts, complexes and prodrugs thereof; e.g. guanosine analogues, deoxyguanosine analogues, guanine, guanine analogues, thymidine analogues, uracil analogues and adenine analogues. Especially interesting antiherpes virus agent for use either alone or in combination in a composition according to the present invention are selected from acyclovir, famciclovir, deciclovir, penciclovir, zidovudin, ganciclovir, didanosin, zalcitabin, valaciclovir, sorivudine, lobucavir, brivudine, cidofovir, n-docosanol, ISIS-2922, and prodrugs and analogues thereof. Details concerning active substances suitable for use in connection with the present invention as well as a description of other interesting active substances are given below.

As mentioned above an important property of a composition according to th present invention is its ability to generate a liquid crystalline phase. The term "liquid crystalline phase" as used herein is used to denote an intermediate state between solid crystals and isotropic liquids,

characterized by long-range order and short-range properties close to those of a simple liquid or solution (Keller et al., Handbook of Liquid Crystals, Verlag Chemie, Weinheim, Germany, 1980).

Examples of fatty acid esters with an excellent ability of forming a liquid crystalline phase are glyceryl monoesters of fatty acids. Specific examples include glycerylmonooleate (monoolein) and glycerylmonolinoleate. Such fatty acid esters are capable of forming various crystalline phases upon contact with a hydrophilic medium such as water or glycerol. As will be explained in further detail below, these fatty acid esters also show so-called bioadhesive properties.

Liquid crystalline phases may be a cubic (three cubic phases are known: i) the body-centred lattice, ii) the primitive diamond lattice, and iii) the gyroid), hexagonal, reverse hexagonal or lamellar phase. By the term "cubic phase" herein is meant a thermodynamically stable, viscous and optically isotropic phase made of a fatty acid ester and an aqueous medium. The term "aqueous medium" includes media containing water or another hydrophilic and water-miscible substance such as, e.g., glycerol. The terms "hexagonal phase" and "reverse hexagonal phase", respectively, are used herein to describe thermodynamically stable, viscous and optically anisotropic phases characterized by long-range order in two dimensions and made of a fatty acid ester and an aqueous medium. The term "lamellar phase" is characterised by a long-range order in one dimension. The lamellar structure is the origin of liposomes having spherical shells of lipid bilayers. The various liquid crystalline phases can be detected and identified by use of polarized light or by means of X-ray diffraction pattern analysis (see the Examples herein). The cubic phase is normally the preferred phase in the compositions of the invention, but also, e.g., the reverse hexagonal phase may be an interesting liquid crystalline phase in the compositions according to the invention.

In accordance with the above-mentioned observations, a fatty acid ester for use according to the present invention may be a fatty acid ester which is capable of forming a liquid crystalline phase on contact with a suitable liquid phase. The liquid of the liquid phase is suitably water or an aqueous medium. An aqueous medium is a medium containing water at least in part.

Apart from aqueous solutions or dispersions such a medium with which the liquid crystalline phase is made may, especially for the precursor embodiment of the composition, at least in part be constituted by any body fluid or secretion which contains water and with which the composition comes into contact upon application, such as, e.g. in the case of a human body fluid, saliva, sweat, gastric juice, etc. As indicated above, the body liquid may induce formation of a liquid crystalline phase when a fatty acid ester is contacted with such a liquid.

However, in many embodiments, the composition according to the invention will be one in which the liquid crystalline phase is already present, that is, the liquid crystalline phase has already been established by interaction between the liquid phase and the fatty acid ester. In this case, the liquid of the liquid phase may, e.g., typically be water or glycerol or a mixture thereof, water often being a preferred liquid.

As mentioned above, the active substance of the composition of the invention is one whose solubility in the liquid crystalline phase is low, at the most 20 mg/g at 20°C, such at the most 15 mg/g at 20°C, e.g. at the most 10 mg/g at 20°C or lower, such as at the most 7 mg/g, 6.5 mg/g, 6 mg/g, 5..5 mg/g, 5 mg/g at 20°C. e.g. at the most 4 mg/g at 20°C or even at the most 3 mg/g or 2 mg/g or 1 mg/g at 20°C.

The determination of the solubility of the active substance in the liquid crystalline phase of the composition is, of course, performed on the liquid crystalline phase as formed. In practice, this means that when the composition is one in which the liquid crystalline phase has already been formed when the composition is applied, the determination of the solubility is performed on the composition itself. The determination of the solubility is suitably performed by microscopy to observe any crystals of the active substance. The determination of the concentration at which crystals are observed is performed after a period of at least one week after preparation of the composition or the liquid crystalline phase, or when equilibrium has been established. Normally, a series of tests with varying concentrations is performed to determine the concentration above which crystals are found. On the other hand, when the composition is a precursor composition, the liquid crystalline phase used as a reference in the solubility determination is a liquid crystalline phase imitating the liquid crystalline phase which will be formed when the composition absorbs liquid from the site of application. This reference liquid crystalline phase is made up with water (as representing the liquid absorbed) in such an amount that the reference liquid crystalline phase is the same type of liquid crystalline phase as is generated from the precursor composition.

While the lower limit of the amount of the fatty acid ester in the composition is determined by the requirement that the fatty acid ester, in the amount in question, must be able to form and maintain the liquid crystalline phase, the composition will in most cases contain at least 20% by weight, calculated on the composition, of the fatty acid ester, normally at least 30% by weight, and in most cases preferably at least 40% by weight, calculated on the composition, of the fatty acid ester. These numbers apply to the liquid crystalline phase pres nt in th composition; in precursor compositions, the concentrations will, of course, be higher.

The pH of the liquid crystalline phase of the composition is in the range of 3.6-9. At lower pH values, the composition may be irritating to the skin or mucosa on which it is applied; at higher pH values, the composition may be irritating and may also directly be etching. The pH of the liquid crystalline phase is determined by a method involving dispersing e.g. 10% of the liquid crystalline phase (containing the active substance and any excipients) in distilled water and measuring the pH in the water phase, equilibration between the liquid crystalline phase and a water phase and measuring the pH of the water phase at 20°C. Alternatively, the pH of the liquid crystalline phase may be measured by means of an suitable pH electrode (see the Examples).

It is generally preferred that the upper limit of the pH of the liquid crystalline phase is 8. It is also preferred that the lower limit of the pH is 3.6 or higher, and thus, interesting pH ranges for the liquid crystalline phase are pH 3.6-8, such as 3.7-8, e.g. 3.8-8, such as 3.9-8, e.g. 4.0-8, such as 4.1-8, eg. 4.2-8, e.g. 4.3-8, such as 4.5-8, e.g. 4.75-8, such as 5.0-8.

As stated above, the solubility of the active substance in water is very low, at the most 10 mg/g at 20°C and at a pH substantially identical to the pH of the liquid crystalline phase, determined as described herein. While a pH range is stated above for the liquid crystalline phase, it will be understood that by the water solubility of the active substance is meant the water solubility at the relevant pH, which is a pH substantially identical to the pH which will prevail in the composition, in other words, the pH of the liquid crystalline phase, this pH being determined as described herein. When the pH of the liquid crystalline phase, determined as described herein, is different from the pH which will result simply by dissolution of the active substance in water, the water is adjusted to substantially the pH of the liquid crystalline phase by using a suitable buffer system when determining the solubility of the active substance. This buffer system should of course be so selected that, apart from the pH adjustment, it has substantially no influence on the solubility of the active substance in the buffered water.

The composition according to the present invention is very valuable in that it can provide a high release of active substances of very low water solubility, such as
a solubility of at the most 7 mg/g, such as at the most 5 mg/g at 20°C and at a pH substantially identical to the pH of the liquid crystalline phase, determined as described herein.

Of particular interest is also the fact that excellent release rates can be obtained of active substance whose solubility in water is at the most 3 mg/g or even at the most 2 mg/g at 20°C and at a pH substantially identical to the pH of the liquid crystalline phase, determined as described herein.

Alternatively, the active substance has an minimum aqueous solubility of at the most 10 mg/ml such as, e.g., 7 mg/ml, 5 mg/ml, 3 mg/ml and 1 mg/ml at 20°C and at a pH in a range corresponding to 3.6-9. The determination of the minimum aqueous solubility is performed by use of suitable buffers which are capable of maintaining the pH at the desired value and measures are taken to ensure that equilibrium is obtained between the undissolved and dissolved active substance, i.e. by employment of ultrasonic treatment and/or stirring for a well-defined time period. It will be appreciated that the pH-ranges and the aqueous solubility values given above when the aqueous solubility is determined at a pH corresponding to the pH prevailing in the liquid crystalline phase apply mutatis mutandis when the aqueous solubility is the minimum solubility in a pH range of 3.6-9.

In embodiments of particular interest a composition according to the invention contains one or more antiherpes virus agent(s) as an active substance. Relevant antiherpes virus agents are mentioned above and acyclovir is of particular importance. Acyclovir (9-[2-hydroxyethoxy)methyl]-guanine, an acyclic analogue to the natural nucleoside 2'-deoxyguanosine, is a widely used agent in the treatment of herpes virus infections. Compositions for oral, topical and intravenous administration are available. The delivery characteristics of acyclovir following administration by these routes are, however, far from being optimal probably due to the poor aqueous solubility and/or low lipophilicity of acyclovir. The solubility of acyclovir in water is about 1.5 mg/ml at 22°C and the partition coefficient (P) between octanol and 0.02 M phosphate buffer pH 7.4 (21°C) is about 0.03. In accordance with the physico-chemical properties, the bioavailability after oral administration is rather low (about 15-20%) and highly variable and the percutaneous penetration is poor.

With respect to acyclovir, it is believed that a composition with improved release properties and which sticks better to the skin can improve the treatment when compared to prior art compositions such as Zovir® or Zovirax®. The object of the present invention has therefore inter alia been to develop a bioadhesive composition containing e.g. acyclovir or other antiherpes virus agents with improved release properties so that fewer daily applications are needed to produce the same therapeutic effect (bioequivalence) or even improve the therapeutic effect.

As appears in more detail in the Experimental section herein, the present inventors have developed compositions containing GMO/water 65/35% w/w with acyclovir (crystalline and micronized, respectively) added in a concentration of 1-40% w/w. Cubic phases are obtained in these compositions as evidence by polarized light. The results indicate that acyclovir in the concentration range investigated does not ruin the cubic lattice, and that acyclovir probably is inert in the cubic system. The distribution of the drug crystals in the cubic phase appears as a homogeneous distribution (observed by microscopy). The cubic phase without drug is

transparent and has a relatively high viscosity. It is cosmetically appealing. When acyclovir is added, th viscosity is increased with the concentration, esp cially for the micronized quality. When the crystalline quality is added, the composition becomes greyish white. When the cubic phase is applied to human skin it melts and penetrates the skin.

As mentioned above, Zovir® and Zovirax® cream containing 5% w/w acyclovir are presently the drugs of choice for the treatment of herpes simplex. In order to compare the release rate of acyclovir from Zovir® cream and a cubic phase (GMO/water 65/35 % w/w) containing 5% w/w acyclovir, the release of acyclovir from these compositions was examined, cf. Example 16 herein. Comparing the rate constants it is seen that the release rate of acyclovir is about 5-6 times faster from the cubic phase than from the Zovir® cream. Poor release properties of the Zovir® cream are most likely one of the reasons for its suboptimum therapeutic effect. The improved release properties from the cubic phase must therefore be seen as a very promising result.

Important embodiments of the present invention are compositions in which the active substance is present in a concentration which is above the saturation concentration at 20°C so that part of the active substance, and in many cases the predominant proportion of the active substance, is present in the form of particles, such as, e.g., crystals. In such a case, normally at least 25%, such as at least 50%, by weight of the active substance present in the composition constitutes a proportion which is present above the saturation concentration at 20°C. Very valuable compositions according to the invention are compositions, wherein at least 75%, such as at least 90% or even at least 95% or at least 98% by weight of the active substance present in the composition constitutes a proportion which is present above the saturation concentration at 20°C.

While the present invention is not to be limited to any theory, it is believed, and supported by experimental data reported herein, that the capability of the composition to release the active substance of very low water solubility and very low solubility in the liquid crystalline phase at very satisfactory release rates is due to some kind of efficient dissolution system for particles, such as crystals, of the active substance through the liquid phase "channels" of the liquid crystalline phase.

The fatty acid esters capable of generating a liquid crystalline phase as evidenced by one of the test methods described herein are fatty acid esters (i.e. composed of a fatty acid component and a hydroxy-containing component) wherein the fatty acid component of the fatty acid ester is a saturated or unsaturated fatty acid having a total number of carbon atoms of from C_6 to C_{26}.

Specific examples of saturated fatty acid moieties in the fatty acid esters according to the inventi n are selected from the group consisting of moieties of caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, and behenic acid.

Specific examples of unsaturated fatty acid moieties in the fatty acid esters according to the invention are moieties selected from the group consisting of palmitoleic acid, oleic acid, linoleic acid, linolenic acid, and arachidonic acid.

Particularly suitable fatty acid esters for use according to the invention are fatty acid esters which are selected from the group consisting of fatty acid esters of polyhydric alcohols, fatty acid esters of hydroxycarboxylic acids, fatty acid esters of monosaccharides, fatty acid esters of glycerylphosphate derivatives, fatty acid esters of glycerylsulfate derivatives, and mixtures thereof. In those cases where the hydroxy-containing component of the fatty acid ester is polyvalent, the hydroxy-containing component may be partially or totally esterified with a fatty acid component or with mixtures of fatty acid components.

The polyhydric alcohol component of the fatty acid ester for use according to the invention is preferably selected from the group consisting of glycerol, 1,2-propanediol, 1,3-propanediol, diacylgalactosylglycerol, diacyldigalactosylglycerol, erythritol, xylitol, adonitol, arabitol, mannitol, and sorbitol. The fatty acid esters formed from such polyhydric alcohols may be mono- or polyvalent such as, e.g., divalent, trivalent, etc. In particular fatty acid monoesters have proved to have bioadhesive properties and are therefore preferred fatty acid esters for use according to the invention. The position of the polyvalent alcohol on which the ester bond(s) is(are) established may be any possible position. In those cases where the fatty acid ester is a diester, triester, etc. the fatty acid components of the fatty acid ester may be the same or different. In a most preferred aspect of the present invention, the polyhydric alcohol component is glycerol.

Examples of fatty acid esters for use according to the invention and wherein the hydroxy-containing component is a polyhydric alcohol are glycerylmonooleate, glycerylmonolinoleate, glycerol monolinoleate, and mixtures thereof. These fatty acid esters have especially promising bioadhesive properties, confer the Examples herein.

In those cases where the fatty acid ester for use according to the present invention is formed between a hydroxycarboxylic acid (or a derivative thereof) and a fatty acid (or a derivative thereof), the hydroxycarboxylic acid component of the fatty acid ester is preferably selected from the group consisting of malic acid, tartaric acid, citric acid, and lactic acid. An interesting exampl of a fatty acid ester for use according to the invention is a fatty acid monoester of citric acid.

As mentioned above, the hydroxy-containing component of a fatty acid ester for use according to the present invention may also b a saccharide, such as a monosaccharide such as, e.g., glucose, mannose, fructose, threose, gulose, arabinose, ribose, erythrose, lyxose, galactose, sorbose, altrose, tallose, idose, rhamnose, or allose. In those cases where the hydroxy-containing component is a monosaccharide, the fatty acid ester is preferably a fatty acid monoester of a monosaccharide selected from the group consisting of sorbose, galactose, ribose, and rhamnose.

The hydroxy-containing component of a fatty acid ester for use according to the invention may also be a glycerylphosphate derivative such as, e.g., a phospholipid selected from the group consisting of phosphatidic acid, phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositole, and diphosphatidylglycerol.

Especially interesting compounds having a phospholipid moiety are compounds wherein the fatty acid ester is a fatty acid ester of a glycerylphosphate derivative, and the fatty acid component is selected from the group consisting of lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, and behenic acid. Examples of such useful fatty acid esters are dioleyol phosphatidylcholin, dilauryl phosphatidylcholin, dimyristyl phosphatidylcholin, dipalmitoyl phosphatidylcholin, distearoyl phosphatidylcholin, dibehenoyl phosphatidylcholin, dimyristyl phosphatidylethanolamine, dipalmitoyl phosphatidylethanolamine, dioleyl phosphatidylglycerol, dilauryl phosphatidylglycerol, dimyristoyl phosphatidylglycerol, dipalmitoyl phosphatidylglycerol, distearoyl phosphatidylglycerol, dipalmitoyl phosphatic acid and mixtures thereof.

Most of the fatty acid esters for use according to the invention are well-known chemical compounds which are commercially available or may be prepared by means of conventional esterification procedures involving e.g. reaction of a fatty acid derivative such as, e.g., the corresponding acid chloride with a hydroxy-containing compound (if necessary protected with suitable protection groups) and subsequently isolating the fatty acid ester, if necessary after removal of any protecting group. Many of the commercially available fatty acid esters are employed in the food industry and in general, no steps are taken in order to obtain an approximately 100% pure fatty acid ester. As an example it can be mentioned that glycerylmonooleate from Grindsted Products A/S, Denmark is a very pure product containing about 98% w/w monoesters of which more than about 80% w/w (such as about 92% w/w) is glycerylmonooleate; the remaining monoesters are glycerylmonolinoleate, glyceryl monopalmitate and glyceryl monostearate. The fatty acid ester products for use according to the invention may thus be mixtures of fatty acid esters.

Examples of fatty acid esters with excellent bioadhesive properties as well as an excellent ability of forming a liquid crystalline phase are glyceryl monoesters of fatty acids. Specific examples include glycerylmonooleate (monoolein) and glycerylmonolinoleate. As mentioned above, such fatty acid esters are capable of forming various crystalline phases upon contact with a hydrophilic medium such as water or glycerol, a preferred liquid crystalline phase being the cubic phase.

Thus, very interesting compositions according to the invention are compositions in which the fatty acid ester is glycerylmonooleate or glycerylmonolinoleate, in particular glycerylmonooleate.

It has been found that the stability of the composition is considerably enhanced, such as resulting in a storage stability of at least 2 years at 20°C, when the glycerylmonooleate product (as is well known, fatty acid esters are almost invariably mixed products) contained in the product fulfils certain purity standards. Thus, the glycerolmonooleate product used for the preparation of the composition should contain at the most 4% of saturated monoglyceride and should preferably contain at least 88% of glycerylmonooleate, more preferably at least 89%, such as at least 90% or at least 91%, in particular at least 92%, of glycerylmonooleate.

When the composition is a precursor type composition, the liquid phase is either not present at all or is present in small amounts, such as an amount of at least 0.5% by weight, such as at least 1% by weight, calculated on the total composition, e.g. at least 2% by weight, calculated on the total composition, or up to at least 5% or in certain cases at least 10%, calculated on the total composition.

In non-precursor compositions, the liquid phase is normally present in an amount of at least 20% by weight, calculated on the total composition, such as at least 25% or at least 30% by weight, calculated on the total composition, and a preferred amount is often in the range of 25-50% such as 30-50% by weight, in particular 27-40%, 27-37% or 30-40% by weight, calculated on the total composition.

The active substance may have any degree of lipophilicity. In certain interesting compositions, the active substance is one which has a lipophilicity of at the most 100, such as at the most, e.g., 75, 50, 25, 10, 7.5, 5 or 2.5, expressed as the partition coefficient between octanol and 0.05M phosphate buffer, pH 7, at 20°C, in some a partition coefficient of at the most 10 or even at the most 1 or at the most 0.75, 0.5, 0.1, 0.075, 0.05 or 0.04.

Alternatively, the lipophilicity may b expressed as the partition coefficient between octanol and an appropriate buffer having a pH corresponding either to the pH of the liquid crystalline phase

or to the pH at which the active substance has its minimum solubility. In such cases, the value mention d above are also valid.

The performance of the compositions according to the invention with respect to releasing the active substance from the liquid crystalline phase can be adequately expressed by the slope of the cumulative release in μg as a function of the square root of the release time in hours in the release experiment defined in connection with Fig. 6 (in which the concentration of the substance is 5%). In preferred compositions according to the invention, the slope is at least 50, more preferred at least 100.

An expression of better performance is a slope of at least 200, such as at least 300, or at least 500 or even at least 700 or at least 900.

As mentioned above, it is a great advantage of the compositions according to the invention that the fatty acid esters can confer bioadhesivity to the compositions. During the last decade increased attention has been given to the possibility of using bioadhesive/mucoadhesive polymers for drug delivery purposes. It is believed that several problems associated with conventional controlled release drug delivery systems may be reduced or eliminated by using a bioadhesive/mucoadhesive drug delivery system. In conventional controlled release drug delivery systems no precautions are made in order to localize the delivery system after administration and, furthermore, the contact time in vivo between the drug delivery system and a particular site is often so short that no advantages are to be expected with respect to, e.g., modifying tissue permeability. Compared with conventional controlled release drug delivery systems, bioadhesive drug delivery systems are believed to be beneficial with respect to the following features:

i) a bioadhesive drug delivery system localizes a drug substance in a particular region, thereby improving and enhancing the bioavailability for drug substances which may have poor bioavailability in themselves,

ii) a bioadhesive drug delivery system leads to a relatively strong interaction between a bioadhesive substance and a mucosa; such an interaction contributes to an increasing contact time between the drug delivery system and the tissue in question and permits localization of the drug delivery system to a specific site,

iii) a bioadhesive drug delivery system is contemplated to prolong delivery of drug substances in almost any non-parenteral route,

iv) a bioadhesive drug delivery system can be localized on a specific site with the purpose of local therapy e.g. treatment of local fungal diseases, permeability modification, protease and other enzyme inhibition, and/or modulation of immunologic expression,

v) a bioadhesive drug delivery system may be targeted to specific diseased tissues, and

vi) a bioadhesive drug delivery system may be employed in those cases where the conventional approach to controlled release drug delivery is unsuitable, i.e. for certain drug substances or classes of drug substances which are not adequately absorbed.

Thus, preferred compositions according to the present invention are compositions in which the fatty acid ester or combination of fatty acid esters present in the composition complies with the requirements of bioadhesion defined herein when tested for bioadhesion in an in vivo model or any other bioadhesivity model as given in the experimental section herein. Especially preferred are compositions which in themselves comply with the requirements of bioadhesion defined herein when tested for bioadhesion in an in vivo model or other bioadhesivity model as given in the experimental section herein.

Thus, interesting compositions are compositions in which the fatty acid ester or combination of fatty acid esters, when tested in a bioadhesive test system, comprising:

i) placing a segment of longitudinally cut rabbit jejunum on a stainless steel support in such a manner that the mucosa layer of the jejunum is placed upside so as to allow application of said fatty acid ester,

ii) placing the resulting support at an angle of -21° ± 2° in a cylindrical cell thermostated at 37°C ± 0.5°C and with the relative humidity kept at about 100%,

iii) flushing the jejunum on the support with 0.02M isotonic phosphate buffer solution (pH 6.5, 37°C) for 5 min at a flow rate of 10 ml/min,

iv) applying an accurately weighed amount of a sample of said fatty acid ester (about 100 mg) on a surface area (about 0.8 x 6 cm) of the mucosa of the jejunum on the support,

v) dropping about 0.5 ml of said phosphate buffer solution on the sample applied,

vi) leaving the resulting sample from step v) for 10 minutes in said cell to allow the sample to interact with glycoproteins of the jejunum,

vii) flushing the jejunum with the sample applied with said phosphate buffer solution (pH 6.5, 37°C) for 30 minutes at a flow rate of 10 ml/min,

viii) collecting the washings resulting from step vii), and

ix) calculating the residual amount of the sample remaining on the jejunum by measuring the amount of the sample in the washings or by measuring the amount remaining on the jejunum,

results in a residual amount of at least 60% w/w, in particular a residual amount of at least 70% w/w, such as at least 80% w/w, preferably at least 85% w/w and more preferably at least 90% w/w.

Interesting compositions are also compositions as defined further above which, when tested in the jejunum test system defined in claim above, result in a residual amount of at least 40% w/w of the fatty acid ester or combination of fatty acid esters or at least 40% w/w of the active substance.

A measure of the bioadhesivity of a composition itself is that it complies with the requirements for bioadhesion defined herein when tested for bioadhesion in the in vivo model described herein involving testing the rinsing off ability from skin.

The active substance of low solubility is normally present in the composition in an amount in the range of from 1-20% by weight, usually 1-15% by weight.

As mentioned above, an important example of an active substance is an antiviral drug, such as a nucleoside or a nucleoside analogue, e.g. selected from acyclovir, famciclovir, deciclovir, penciclovir, zidovudin, ganciclovir, didanosin, zalcitabin, valaciclovir, sorivudine, lobucavir, brivudine, cidofovir, n-docosanol, ISIS-2922 and salts and prodrugs thereof. However, also a large number of other drugs which in themselves have a low solubility as defined herein or the salts, esters, prodrugs or precursors of which have a low solubility are important active substances in the compositions of the invention. Furthermore, there is also a large number of drugs which advantageously can be incorporated in a composition according to the invention, either as the sole active substance (provided the solubility criteria are fulfilled) or in combination with another active substances. In the following is listed a number of active substance which either alone or in combination may be incorporated in a composition according to the present invention. In particular a combination of an antiherpes virus agent and a glucocorticosteroid is of importance.

Examples of drugs which are of particular importance in connection with application to skin or mucosal surfaces ar :

Acyclovir, famciclovir, ribavirin, zidovudin, ganciclovir, didanosin, zalcitabin, valaciclovir

amantadin, rimantadin

foskarnet

idoxuridin
fluoruracil

interferons and variants thereof, including alpha interferon, beta interferon, and gamma interferon,

tromantadin

lentinan
levofloxacin
stavudine
tacrine
vesnarinone
ampligen

atevirdine
delavirdine
hydroxyurea
indinavir sulfate
interleukin-2 fusion toxin, seragen
lamivudine
lidakol
nevirapine
onconase
saquinavir
topotecan
verteporfin
viraplex

CMV immunoglobulin
efalith
epervudine
podophyllotoxin
proxigermanium
rifabutin
bromovinyldeoxyuridine
ukrain
cidofovir
imiquimod
lamivudine
sorivudine
viraplex
afovirsen
amonafide
hypericin

provir
temoporfin
aphidicolin glycinate
ibobucavir
virend

AL-721
ampligen
arildone
brivudine
CD4
2-deoxy-D-glucose
desciclovir
dichloroflavan
didanosine
ditiocarb Sodium
edoxudine
enviroxime
fiacitabine
inosine Pranobex
peptide T

stavudine
tribavirin
trifluridine
vidarabine
zalcitabine

miconazol
fucidin
erythromycin
macrolides
NSAID's
peptides
insulin
polymycin
myperizin
antibiotics
nicotine
sucralfate
sucrose octasulfate
salicylic acid
urea
benzoylperoxide
minoxidil
heparinoid
methotrexate
ciclosporin

A listing of substances of potential interest comprises substances of the following groups:

anti-inflammatory drugs such as, e.g., ibuprofen, indomethacin, naproxen, diclofenac, tolfenamic acid, piroxicam, and the like;

narcotic antagonists such as, e.g., naloxone, nalorphine, and the like;

antiparkinsonism agents such as, e.g., bromocriptine, biperidin, benzhexol, benztropine, and the lik ;

antidepressants such as, e.g., imipramine, nortriptyline, pritiptylene, and the like;

antibiotic agents such as, e.g., clindamycin, erythromycin, fusidic acid, gentamicin, mupirocien, amfomycin, neomycin, metronidazole, silver sulphadiazine, sulphamethizole, bacitracin, framycetin, p lymycin B, acitromycin, and the like;

antifungal agents such as, e.g., miconazol, ketoconazole, clotrimazole, amphotericin B, nystatin, mepyramin, econazol, fluconazol, flucytocine, griseofulvin, bifonazole, amorolfine, mycostatin, itraconazole, terbenafine, terconazole, tolnaftate, and the like;

antimicrobial agents such as, e.g., metronidazole, tetracyclines, oxytetracycline, and the like;

antiemetics such as, e.g., metoclopramide, droperidol, haloperidol, promethazine, and the like;

antihistamines such as, e.g., chlorpheniramine, terfenadine, triprolidine, and the like;

antimigraine agents such as, e.g., dihydroergotamine, ergotamine, pizotyline, and the like;

coronary, cerebral or peripheral vasodilators such as, e.g., nifedipine, diltiazem, and the like;

antianginals such as, e.g., glyceryl nitrate, isosorbide denitrate, molsidomine, verapamil, and the like;

calcium channel blockers such as, e.g., verapamil, nifedipine, diltiazem, nicardipine, and the like;

hormonal agents such as, e.g., estradiol, estron, estriol, polyestradiol, polyestriol, dienestrol, diethylstilbestrol, progesterone, dihydroergosterone, cyproterone, danazol, testosterone, and the like;

contraceptive agents such as, e.g., ethinyl estradiol, lynestrenol, etynodiol, norethisterone, mestranol, norgestrel, levonorgestrel, desogestrel, medroxyprogesterone, and the like;

antithrombotic agents such as, e.g., heparin, warfarin, and the like;

diuretics such as, e.g., hydrochlorothiazide, flunarizine, minoxidil, and the like;

antihypertensive agents such as, e.g., propanolol, metoprolol, clonidine, pindolol, and the like;

corticosteroids such as, e.g., beclomethasone, betamethasone, betamethasone-17-valerate, betamethason -dipropionate, clobetas l, clobetas l-17-butyrate, clobetas l-propionate, desonide,

desoxymethasone, dexamethasone, diflucortolone, flumethasone, flumethasone-pivalate, fluocinolone acetonide, fluocinonide, hydrocortisone, hydrocortisone-17-butyrate, hydrocortisone-buteprate, methylprednisolone, triamcinolone acetonide, budesonide, halcinonide, fluprednide acetate, alklometasone-dipropionate, fluocortolone, fluticason-propionate, mometasone-furate, desoxymethasone, diflurason-diacetate, halquinol, cliochinol, chlorchinaldol, fluocinolone-acetonid, and the like;

dermatological agents such as, e.g., nitrofurantoin, dithranol, clioquinol, hydroxyquinoline, isotretionin, methoxsalen, methotrexate, tretionin, trioxsalen, salicylic acid, penicillamine, and the like;

steroids such as, e.g., estradiol, progesterone, norethindrone, levonorgestrol, ethynodiol, levenorgestrel, norgestimate, gestanin, desogestrel, 3-keton-desogestrel, demegestone, promethoestrol, testosterone, spironolactone, and esters thereof,

nitro compounds such as, e.g., amyl nitrates, nitroglycerine and isosorbide nitrates,

opioid compounds such as, e.g., morphine and morphine-like drugs such as buprenorphine, oxymorphone, hydromorphone, levorphanol, fentanyl and fentanyl derivatives and analogues,

prostaglandins such as, e.g., a member of the PGA, PGB, PGE, or PGF series such as, e.g., misoprostol, dinoproston, carboprost or enaprostil,

a benzamide such as, e.g., metoclopramide, scopolamine,

a peptide such as, e.g., growth hormone releasing factors, growth factors (epidermal growth factor (EGF), nerve growth factor (NGF), TGF, PDGF, insulin growth factor (IGF), fibroblast growth factor (aFGF, bFGF, etc.), and the like), somatostatin, calcitonin, insulin, vasopressin, interferons, IL-2, urokinase, serratiopeptidase, superoxide dismutase (SOD), thyrotropin releasing hormone (TRH), luteinizing hormone releasing hormone (LH-RH), corticotrophin releasing hormone (CRF), growth hormone releasing hormone (GHRH), oxytocin, erythropoietin (EPO), colony stimulating factor (CSF), and the like,

a xanthine such as, e.g., caffeine, theophylline,

a catecholamine such as, .g., phedrine, salbutamol, terbutaline,

a dihydropyridine such as, e.g., nifedipine,

a thiazide such as, e.g., hydrochlorotiazide, flunarizine,

others such as, e.g., propanthelin, silver nitrate, enzymes like Streptokinases, Streptodases, vitamins like vitamin A, tretionin, isotretionin, acitretin, vitamin D, calcipotriol, interferon-α-2b, selen disulfide, pyrethione.

It will be understood that the compositions of the invention may also comprise combinations of active substances, e.g. an active substance together with a potentiator therefor.

As evidenced in the Examples herein, an active or protective substance does not significantly influence the bioadhesive properties of a vehicle provided that the concentration of the active or protective substance is relatively low such as at the most about 10-15% w/w or at the most about 8-10% w/w. The kind of active substance (structure, molecular weight, size, physico-chemical properties, loading, pKa, etc.) will of course be responsible for the maximal concentration which can be incorporated in the vehicle without significantly affecting the bioadhesive properties of the composition. In the Examples herein, it is also demonstrated that the active substance locates in the liquid crystalline phase of the fatty acid ester and most likely the solubility of the active substance in this phase has impact on the bioadhesive properties as well as on the release properties of the composition.

As mentioned above, the application is intended for skin or mucosa. Other applications may of course also be relevant such as, e.g., application on dentures, prostheses and application to body cavities such as the oral cavity. The mucosa is preferably selected from oral, nasal, aural, lung, rectal, vaginal, and gastrointestinal mucosa.

A bioadhesive composition for administration according to the invention may in special cases also be in the form of a multiple unit composition, in the form of, e.g., a powder. A multiple unit composition may be administered to skin or mucosa, preferably the mucosa is selected from oral, nasal, rectal, aural, vaginal, lung, and gastrointestinal mucosa. Most preferred is a bioadhesive composition intended for administration to the gastrointestinal tract.

Bioadhesive compositions according to the invention for application on skin and especially to wounds may in certain cases comprise a polysaccharide in a concentration of at least 15% w/w, calculated on the total weight of the composition. The polysaccharide is preferably selected from the group consisting of carmelose, chitosan, pectins, xanthan gums, carrageenans, locust bean gum, acacia gum, gelatins, alginates, and dextrans, and salts thereof. The compositions are easy to apply on the wound and are believed to be able to extract water from the wound and thereby drying the wound.

Apart from the active r protective substance and the bioadhesive fatty acid ester substance, the bioadhesive compositions for use according to the invention may comprise pharmaceutically or cosmetically acceptable excipients.

The bioadhesive compositions may be in form of, e.g., a spray, a solution, a dispersion, a suspension, an emulsion, powders, gels including hydrogels, pastes, ointments, creams, drenches, delivery devices, suppositories, enemas, implants, aerosols, microcapsules, microspheres, nanoparticles, liposomes, dressings, bandages, plasters, tooth paste, dental care compositions, and in other suitable form.

The bioadhesive compositions may be formulated according to conventional pharmaceutical practice, see, e.g., "Remington's Pharmaceutical Sciences" and "Encyclopedia of Pharmaceutical Technology", edited by Swarbrick, J. & J. C. Boylan, Marcel Dekker, Inc., New York, 1988.

Pharmaceutically acceptable excipients for use in bioadhesive compositions for use according to the invention may be, for example,

inert diluents or fillers, such as sucrose, sorbitol, sugar, mannitol, microcrystalline cellulose, carboxymethylcellulose sodium, methylcellulose, hydroxypropyl methylcellulose, ethylcellulose, starches including potato starch, calcium carbonate, sodium chloride, lactose, calcium phosphate, calcium sulfate or sodium phosphate; and

lubricating agents including glidants and antiadhesives, for example, magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils or talc.

Other pharmaceutically acceptable excipients can be colorants, flavouring agents, plasticizers, humectants, buffering agents, solubilizing agents, release modulating agents, etc.

For application to the rectal or vaginal mucosa suitable compositions for use according to the invention include suppositories (emulsion or suspension type), solutions, enemas, and rectal gelatin capsules (solutions or suspensions). Appropriate pharmaceutically acceptable suppository bases include cocoa butter, esterified fatty acids, glycerinated gelatin, and various water-soluble or dispersible bases like polyethylene glycols and polyoxyethylene sorbitan fatty acid esters. Various additives like, e.g., enhancers or surfactants may be incorporated.

For application to the nasal mucosa, nasal sprays and aerosols for inhalation are suitable compositions for use according to the invention. In a typically nasal formulation, the active ingredients are dissolved or dispersed in a suitable vehicle. The pharmaceutically acceptable

vehicles and excipients and optionally other pharmaceutically acceptable materials present in th comp sition such as diluents, nhancers, flavouring agents, preservatives etc. are all selected in accordance with conventional pharmaceutical practice in a manner understood by the persons skilled in the art of formulating pharmaceuticals.

For application to the oral cavity, teeth, skin or nail, the compositions for use according to the invention may contain conventionally non-toxic pharmaceutically acceptable carriers and excipients including microspheres and liposomes. The formulations include creams, ointments, lotions, liniments, gels, hydrogels, solutions, suspensions, sticks, sprays, pastes, dressings, bandages, plasters, tooth paste, dental care compositions, and the like. The pharmaceutically acceptable carriers or excipients may include emulsifying agents, antioxidants, buffering agents, preservatives, humectants, penetration enhancers, chelating agents, gelforming agents, ointment bases, perfumes and skin protective agents.

Examples of emulsifying agents are naturally occurring gums, e.g. gum acacia or gum tragacanth, naturally occurring phosphatides, e.g. soybean lecithin and sorbitan monooleate derivatives.

Examples of antioxidants are butylated hydroxy anisole (BHA), ascorbic acid and derivatives thereof, tocopherol and derivatives thereof, vitamin E, salts of sulphur dioxide, butylated hydroxy anisole and cysteine.

Examples of preservatives are parabens, such as methyl, ethyl, propyl p-hydroxybenzoate, butylparaben, isobutylparaben, isopropylparaben, potassium sorbate, sorbic acid, benzoic acid, methyl benzoate, phenoxyethanol, bronopol, bronidox, MDM hydantoin, iodopropynyl butylcarbamate, EDTA, propyleneglycol (increases the solubility of preservatives) benzalconium chloride, and benzylalcohol.

Examples of humectants are glycerin, propylene glycol, sorbitol and urea.

Examples of suitable release modulating agents for use according to the invention are glycerol, sesame oil, soybean oil, lecithin and cholesterol.

Examples of penetration enhancers are propylene glycol, DMSO, triethanolamine, N,N-dimethylacetamide, N,N-dimethylformamide, 2-pyrrolidone and derivatives thereof, tetrahydrofuryl alcohol and Azone.

Examples f chelating agents are sodium EDTA, citric acid and phosphoric acid.

Examples of other excipients f r us in compositions for us according to the invention are edible oils like almond oil, castor oil, cacao butter, coconut oil, corn oil, cottonseed oil, linseed oil, olive oil, palm oil, peanut oil, poppyseed oil, rapeseed oil, sesame oil, soybean oil, sunflower oil, and teaseed oil; and of polymers such as carmelose, sodium carmelose, hydroxypropylmethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, chitosane, pectin, xanthan gum, carrageenan, locust bean gum, acacia gum, gelatin, and alginates, and solvents such as, e.g., glycerol, ethanol, propylene glycol, polyethylene glycols such as PEG 200 and PEG 400, Pluronic, polysorbate, and ethylene glycol.

Examples of ointment bases are beeswax, paraffin, cetyl palmitate, vegetable oils, sorbitan esters of fatty acids (Span), Carbopol, polyethylene glycols, and condensation products between sorbitan esters of fatty acids and ethylene oxide, e.g. polyoxyethylene sorbitan monooleate (Tween).

A most important composition according to the invention is one in which the antiviral substance is acyclovir. Examples of important embodiments hereof and of other compositions according to the invention containing nucleosides of low solubility as defined herein are claimed in claims 75-91 and are described in detail in the Examples.

Description of the drawing

Fig. 1 shows a schematic diagram of the apparatus used in the test method denoted test method 1 described in detail in the experimental section herein. The reference numbers illustrate the following:

1. Thermostatic water flow (40°C)
2. Reservoir containing the washing solution (37°C)
3. A peristaltic pump
4. A stainless steel support
5. A model membrane
6. Receiver for collecting the washings

Fig. 2A shows a schematic diagram of the apparatus used in the test method denoted test method 2 described in detail in the experimental section herein. The reference numbers illustrate the following:

1. Instrument probe
2. Stationary plate
3. A first holder
6. Sliding stand
7. Displacement transducer
8. Control unit

4. A model membrane
5. A second holder
9. Personal computer

Fig. 2B shows a schematic diagram of a variation of the apparatus used in the test method denoted test method 2 described in detail in the experimental section herein. The reference numbers illustrate the following:

1. Instrument probe
2. Stationary plate
3. A first holder
4. A model membrane
5. A second holder
6. A thermostatically controlled heater/stirrer
7. A vessel
8. Sliding stand
9. Displacement transducer
10. Control unit
11. Personal computer

Fig. 3 illustrates the pH-solubility profile for acyclovir.

Fig. 4 shows a thermogram indicating the phase transition L_α-to-Q (lamellar to cubic) for a GMO/water composition (85/15% w/w)

Fig. 5 shows the cumulative release of acyclovir (test conditions as described under Fig. 6)

Fig. 6 shows the release of acyclovir (1-5% micronized) delivered from a cubic phase (GMO/water 65/35% w/w) and Zovir® cream, respectively, into isotonic 0.05 M phosphate buffer solution, pH 6.5 (37°C) [% acyclovir released as a function of time]

Fig. 7 shows a Higuchi plot of the release of acyclovir (test conditions as described under Fig. 6)

Fig. 8 shows the release of acyclovir (1%) delivered from GMO/water 65/35% w/w into isotonic 0.05M phosphate buffer solution, pH 6.5 (37°C). A comparison of the release from 1% of micronized acyclovir and 1% of crystalline acyclovir shows that there is no significant difference in the release of the two different qualities of acyclovir using 1% acyclovir

Fig. 9 shows the release of acyclovir (1%) delivered from GMO/water/lecithin 55/35/10% w/w into isotonic 0.05M phosphate buffer solution, pH 6.5 (37°C). It will be seen that in this case, the crystalline acyclovir is released slightly faster than the micronized acyclovir

Fig. 10 shows the release of acyclovir (5%) delivered from GMO/water 65/35% w/w into isotonic 0.05M phosphate buffer solutions, pH 6.5 (37°C). It will be seen that in this case the micr nized acyclovir is released slightly faster than the crystalline acyclovir

Fig. 11 illustrates the release of acyclovir that is micronized from various GMO formulations containing 1% acyclovir into isotonic 0.05M phosphate buffer solutions, pH 6.5 (37°).

Fig. 12 illustrate the cumulative amount of acyclovir permeated through pig skin; the GMO/water is 65/35% w/w containing 5% acyclovir (for details see Example 20).

MATERIALS

Glycerylmonooleate (monoolein), manufactured by Grindsted Products A/S, Denmark

DIMODAN® GMO-90, a distilled monoglyceride

Chemical and physical data

Monoester content	min. 95%
Diglycerides	max. 3%
Triglycerides	max. 0.2%
Free fatty acids	max. 0.5%
Free glycerol	max. 0.5%
Iodine value	approx. 72
Fatty acid composition:	
Oleic acid	92%
Linoleic	6%
Saturated (C_{16}/C_{18})	2%
Melting point	35-37°C
Antioxidants and synergists added:	
Ascorbyl palmitate	max. 200 ppm
α-Tocoph rol	max. 200 ppm
Citric acid	max. 100 ppm

In th following examples, the term "GMO-90" indicates that the above-mentioned glycerol monooleate product is employed, except where otherwise stated.

Glycerylmonooleate 84% "GMO-84" (monoolein), manufactured by Grindsted Products A/S, Denmark; the product used has a total content of fatty acid monoesters of at least about 96%. The product employed in the examples described herein had the following composition of fatty acid monoesters:

Glycerylmonooleate	about 84% w/w
Glycerylmonolinoleate	about 7% w/w
Glyceryl monopalmitate	about 3% w/w
Glyceryl monostearate	about 4% w/w

In the following examples, the term "GMO 84" indicates that this glycerol monooleate product is employed.

Other commercially available glycerol monooleate products (e.g. Myverol 18-99 and GMOrphic 80 available from Kodak Eastman, U.S.A.) which differ in the composition of fatty acid monoesters compared with the products described above may also be applied.

Glycerylmonolinoleate (Dimodan• LS), manufactured by Grindsted Products A/S; the product used has a total content of fatty acid monoesters of at least about 90% such as about 96% w/w. The product employed in the examples described herein had the following composition of fatty acid monoesters:

Glyceryl monopalmitate	about 6% w/w
Glyceryl monostearate	about 6% w/w
Glycerylmonooleate	about 22% w/w
Glycerylmonolinoleate	about 63% w/w

Other commercial available glycerylmonolinoleate products (such as, e.g., Myverol• 18-92 available from Kodak Eastman, U.S.A.) which differ in the composition of fatty acid monoesters compared with the product described above may also be applied.

Miconazol base available from MedioLast SPA, Milano, Italy
Lidocaine hydrochloride available from Sigma Chemical Co., St. Louis, U.S.A.
Lidocaine base available from Sigma Chemical Co., St. Louis, U.S.A.

Acyclovir (crystalline) available from Chemo Iberica, Spain, e.g. a quality where 90-100% of the crystals have a particle size of less than 100 μm

Acyclovir (micronized) available from Chemo Iberica, Spain, e.g. a quality where 100% of the particles have a particle size under 24 μm and not less than 90% under 12μm

Ethanol available from Danisco A/S, Denmark, complies with the DLS standard (98.8-100% w/w ethanol)

Sesame oil available from Nomeco, Denmark

Soybean oil available from Nomeco, Denmark

Glycerol available from Joli Handel ApS, Denmark

Lecithin Epicuron 200 from Lucas Meyer

Benzyl alcohol available from Merck AG, Germany

Water, purified or distilled water

DEAE-dextran (MW = 500,000) available form Sigma Chemical Co., St. Louis, U.S.A.

Sodium alginate (Sobalg FD 120) available from Grindsted Products A/S, Denmark

Hydroxypropylmethylcellulose (Methocel K15MCR Premium USP) available from Colorcon Limited, U.S.A.

Carbopol 934 available from The BFGoodrich Company, U.S.A.

Vitamin E TPGS (d-α-tocopherylpolyethyleneglycol 1000 succinate) available from Kodak Eastman (in the following designated TPGS)

Aspirin available from Sigma, Chemical Co., St. Louis, U.S.A.

Propylene glycol available from BASF Aktiengesellschaft, Germany

Coulter Multisizer II (Coulter), Malvern 2600 droplet and particle size analyse (for the determination of particle size distribution).

Strölein Areameter and Coulter SA3100 for the determination of the surface area of the particles.

METHODS

Test systems for bioadhesion

1. In vitro test system for bioadhesion by means of rabbit jejunum membranes

The test system for bioadhesion described in the following is a modified system f a method described by Ranga Rao & Buri (Int. J. Pharm. 1989, 52, 265-270).

Male albino rabbits (3-4 kg, New Zealand white rabbit SSC: CPH) were fasted for 20 hours before they were killed by means of a pentobarbital sodium injection. The intestines of the rabbits were dissected and placed in an isotonic 0.9% sodium chloride solution at room temperature (about 18°C). Within 30 minutes the jejunums were cut and washed with 0.9% sodium chloride solution. The lumens were gently rinsed with the saline until the intestines were clean. The jejunums were cut into pieces of about 8-9 cm in length and frozen (-20°C) immediately. The jejunums were stored up to 3 months before use (when performing the test described below it was found that the use of fresh jejunum or, alternatively, jejunum which had been frozen for up to 3 months gave reproducible and significantly similar results). Before testing, the segment of jejunum was gently thawed out.

The segment of the jejunum was cut longitudinally. It was placed on a stainless steel support (a tube of 2 cm in diameter and cut longitudinally at an axis parallel to its centre) with the mucosa layer upside, spread and held in position on the support by the adhesive effect of the jejunum itself. The support with the jejunum was placed at an angle of from about -5° to about -25° such as -7° or -21° (in the Examples the angle applied is denoted "angle" in a cylindrical cell thermostated at 37°C. A schematic illustration of the cell is shown in Fig. 1. The relative humidity in the thermostated cell was kept at about 100%. The jejunum was then flushed with a medium of 0.02M isotonic phosphate buffer solution (pH 6.5, 37°C) for 2 or 5 minutes (in the following denoted "initial rinsing period") at a flow rate of 5 or 10 ml/min (in the following denoted "initial rinsing flow"), respectively, using a peristaltic pump to equilibrate the jejunum with the buffer and to rinse off loose mucosa. [Immediately before application of the sample, the support was positioned at a horizontal position and after application the position was changed to the initial position of -21°.] An accurately weighted amount of the sample to be tested for bioadhesive properties (about 50-150 mg) was placed evenly on the mucosa of the jejunum (about 0.8 x 6 cm). About 1 ml of the buffer solution was carefully dropped evenly on the sample applied to ensure formation of such a liquid crystalline phase, if possible (in the case of monoolein, the liquid crystalline phase may be the cubic, hexagonal, reverse hexagonal, micellar, or lamellar phase). [In those cases where the viscosity of the test sample are relatively high or where a precipitation has taken place, the test sample is gently melted on a heating plate or in an oven at a temperature of max. 60°C in the case of GMO or GML and cooled to a temperature of at the most about 40°C before application on the rabbit jejunum.] Immediately after, the segments were left for 5-20 minutes such as, e.g., 10 minutes in the cell allowing the sample to interact with the glycoproteins of the jejunum and to prevent drying of the mucus. After 10 minutes, the segments were flushed evenly with the isotonic 0.02M phosphate buffer solution (pH 6.5, 37°C) for 15-60 minutes such as, e.g., 30 minutes at a flow rate of 5-15 ml/min such as 10 ml/min (in the Examples denoted "flow rate"). The tip of the tube carrying the buffer solution was placed 3-4 mm above the jejunum to ensure an even liquid flow over the mucosa. The

washings were collected into a beaker. The amount of bioadhesiv c mponent remaining n the jejunum was calculated either by measuring the amount of sample in the beaker or by measuring the amount of sample remaining in the jejunum by means of a suitable analysis method, e.g. HPLC.

At the end of the experiment, the remaining sample on the jejunum was checked with a pair of tweezers to reveal false positive results.

In 1-2 test run(s) out of 10, false negative results were observed probably due to a loose mucosa layer on the rabbit jejunum.

During testing and validation of the method, the parameters given above were varied (e.g. the angle applied, the flow rate, the amount applied, etc.). In order to exclude false negative and false positive results it was found that the following conditions were satisfactory:

Time for prehydration before application of sample: 10 min

Amount applied:	about 50-150 mg (tests have shown that a variation in the amount applied within a range of from about 25 mg to about 225 mg was without significant influence on the results obtained)
Angle:	-21°
Flow rate:	10 ml/min
Flow period:	30 minutes (it was found that a flow period of at least 10 minutes gives reproducible results and a prolongation of the period to about 60 minutes does not significantly change the result)

Furthermore, it was found advantageous that the method allows rinsing of the sample applied on the jejunum by an aqueous medium, thus allowing a liquid crystalline phase to be formed. The method also permits application of fluid samples and pellets.

Determination of the bioadhesiveness of a test sample

In those cases where the test sample is a fatty acid ester, the fatty acid ester is considered as bioadhesive if the residual amount is at least about 60% w/w such as at least about 65% w/w, about 70% w/w, about 75% w/w, about 80% w/w, about 85% w/w, 90% w/w, or about 95% w/w.

In those cases where the test sample is a composition comprising a combination of a fatty acid ester and an active or protectiv substance, the composition is considered bioadhesive if the residual amount (of fatty acid ester or active/protective substance) is at least about 40% w/w such as at least about 45% w/w, about 50% w/w, 55% w/w, 60% w/w, 65% w/w, 70% w/w, 75% w/w, or 80% w/w.

In the present context evaluation of the bioadhesive properties of a substance may also be performed by use of the test system and test conditions described above but modified with respect to type of membrane, amount applied of test sample, test angle, flow rate, medium, etc. In this connection, tests have been performed in order to evaluate the influence of different membranes on the test results. The following results were obtained using the above-mentioned test conditions (angle: -21°, flow rate: 10 ml/min, and flow period: 30 min) and applying GMO on the membrane:

Membrane	Bioadhesion
% w/w	Residual amount %
rabbit jejunum	90
pig ileum	106*
pig stomach	106*
buccal pig mucosa	88

* the high result is most likely due to an interference from the intestines or the stomach

2. In vitro test system for bioadhesion by means of tensiometry

The test system for bioadhesion described in the following is a modified system of a method described by Tobyn, M., J. Johnson & S. Gibson (in "Use of a TA.XT2 Texture Analyser in Mucoadhesive Research", International LABMATE, 1992, XVII (issue VI), 35-38).

The test system involves measuring the tensile force required to break an adhesive bond formed between a model membrane and a test sample (i.e. the sample which is tested for its bioadhesive properties).

The test apparatus employed in the following is a TA.XT2 Texture analyser (Stabl Micro System Ltd., Haslemere, UK) (Fig. 2) equipped with a 5 kg load cell interfaced with an IBM PC computer running XT-RA dimension software, DOS version. The test enables measuring the

strength of adhesive bonding established by contacting a model membrane, i.e. in this case a pig intestine segment, and the test sample. An analogous test apparatus may also be employed.

The TA.XT2 Texture analyser apparatus is equipped with an instrument probe 1 (see Fig. 2) which is movable in a vertical direction at a variable rate. During the so-called withdrawal phase of the testing, the instrument probe is moved upwards with a constant rate until detachment occurs (see below). Furthermore, the apparatus is equipped with a stationary plate 2 on which a first holder 3 is placed. Before and during a test run, a model membrane 4 is fixed on this holder, e.g. by means of a cap or double adhesive tape or glue. The area exposed to the test may be determined by the area of the probe (preferred in this case) or by the area of the test samples (e.g. a coated cover glass), or by the area of a holder fixed to the probe. The accurate size of the exposed area is used in the calculation of the adhesive strength (see below).

As mentioned above, the test involves employment of a model membrane, primarily of animal origin. The membrane could be e.g. rabbit, rat or pig gastric mucosa; a segment of rabbit, rat or pig intestines, e.g. a segment of rabbit jejunum; a segment of rabbit or porcine buccal mucosa; or a segment of rabbit, rat or pig intestines from which the mucosal layer has been removed prior to testing; or skin from an animal (after removal of substantially all subcutaneous fat); or it could be artificially or commercially available mucin.

In the tests described below, duodenum, jejunum and the upper part of ileum from freshly slaughtered pigs were used. The gut was stored on ice until it was washed with 0.9% w/w sodium chloride solution within 2 hours. The lumens were gently rinsed with the saline until the intestines were clean. The gut was cut into pieces of 3-4 cm and immediately frozen (-20°C). The intestines were stored up to 2 months before use. Before testing, the segments were gently thawed out. The gut segment was opened along the mesenteric border. Serosa and muscularis layers were removed by stripping with a pair of tweezers, taking care to maintain the integrity of the mucus layer. This resulted in a flattening of the originally folded mucosal surface. Before use the tissue was equilibrated in the testing medium for about 10 min, which was sufficient for the tissue to attain temperature and pH equilibrium as measured by pH paper.

If the results obtained by use of another membrane than the one mentioned above are compared to the bioadhesive properties of various substances or combinations, the results of a reference compound could be included. As discussed below testing of a reference sample may also be made as a routine. Polycarbophil and Carbopol 934 have been found suitable as reference compounds.

An accurate amount of a test sample (about 25-500 mg) is applied in a uniform layer eith r

i) on the luminal side of the model membrane placed on the first holder,

ii) directly on the instrument probe, if necessary by means of a cap, a double adhesive tape or glue applied on the instrument probe before application of the test sample,

iii) on a cover glass which is placed on the instrument probe with the test sample pointing downwards, or

iv) via a probe modified in such a manner that it allows application of a relatively low viscous or semi-solid sample, the modified probe also allows the necessary addition of an aqueous medium.

In those cases where it is not possible to fix the test sample to the instrument probe, the apparatus may be equipped with a second holder 5 on which another model membrane is fixed. In such cases, the model membranes employed on the two holders are usually of the same type. It is also possible to fix the other model membrane directly to the instrument probe e.g. by means of a double adhesive tape, glue, or a cap.

For an adhesion test, a tissue (porcine intestinal mucosa) of about 3 x 3 cm was fixed on the tissue holder 3 with the mucosa layer upside. Before application of the tissue, a piece of gauze was placed directly on the tissue holder, and thereupon the tissue was placed. This precaution is made in order to stabilize the contact force. In order to moist the tissue and hydrate the sample, about 0.5 ml isotonic 0.05M phosphate buffer, pH 6.0, was added to the tissue. Such an addition also enables a cubic phase to be formed. The instrument probe with sample (e.g. applied by smearing 50-80 mg of the sample onto the probe in a thin, smooth layer, see below) was lowered with a test speed of 0.1 mm/sec in order to bring the tissue and the sample in contact under a constant force. The contact area was either 1.33 cm^2 (cover glass) or 1.27 cm^2 (probe) depending on the method of sample preparation. The contact force was set to 0.2N and the contact time was 30 min. After 30 min the probe was withdrawn with a rate of 0.1 mm/sec (post test speed) for 10 mm. Initial experiments showed that this distance was well beyond the point where the sample and mucous separated during withdrawal.

The peak detachment force and the area under the force/time curve was calculated automatically using the XT-RA dimension software. The work of adhesion (mJ cm^{-2}), said to be the most accurate predictor of mucoadhesive performance, was calculated.

Sample preparation

Application method of the polymers used as reference:

Cover glasses having a diameter of 13 mm (area 1.33 cm^2) were coated with the polymers under investigation by pipetting 100 µl of a 1% w/w solution of methanol or water in the center of the glass plate. After drying for 2 hours at 60°C in an oven, a thin polymer film remained. One cover glass was attached to the probe (diameter of 12.7 mm) with its non-coated side by means of double adhesive tape.

Cover glasses and mucosa were only used once (i.e. for one measurement).

Application of fatty acid ester compositions:

A. Melting (if possible) of the solid or semi-solid composition and dipping the probe into it (this method is only used if the melting procedure does not change the properties of the composition). The sample (25-100 mg) was applied to the probe in a smooth layer by dipping the probe into melted GMO. The sample was solidified at room temperature or, if necessary, by cooling.

B. Smearing 25-100 mg of the sample directly on the probe.

C. Fixing the sample by means of a cap, double adhesive tape, or glue

Test runs are performed after the tissue has equilibrated in an aqueous medium at room temperature for 5-20 min. Then the tissue was removed from the aqueous medium and placed in the test apparatus and then the test was run.

In some cases, variations of the above-given method may be relevant, e.g. running the test in an aqueous medium or running the test at a temperature different from room temperature such as 37°C.

Furthermore, the test parameters may be varied, e.g. as follows:

Hydration time:	0 - 20 min
Contact time:	60 sec - 50 min
Contact force:	0.05-0.4N
Equilibration medium	
Test speed:	0.02-1 mm/sec
Post test sp ed:	0.02-1 mm/sec

Test run temperature may be changed by employing a suitable temperatur controlled oven such as a SMTC/04 from Stable Microsystems, Haslemere, UK.

Determination of the bioadhesive properties of a test sample

In order to test whether a test sample is bioadhesive, two test runs are performed:

1. A test run with the test sample applied (result: work of adhesion WA_S),

2. A test run with a known and excellent bioadhesive sample (e.g. polycarbophil) (result: work of adhesion WA_R).

In both cases the work of adhesion is calculated and the test sample is considered bioadhesive if WA_S/WA_R x 100% is at least 30%, such as 35%, 40%, 45%, 50%, or 55%. In general, a sample is graded to be a weak bioadhesive if the result is less than about 30%, a medium bioadhesive if the result is about 30%-50%, a strong bioadhesive if the result is at least 50%.

Polycarbophil (Noveon™ AA-1, BF Goodrich, Hounslow, U.K.) is a high molecular weight poly(acrylic acid)copolymer loosely cross-linked with divinyl glycol. On account of its known excellent mucoadhesive properties, this polymer serves as a reference. Before testing in the above-mentioned tensiometric test, a polycarbophil gel is prepared by mixing polycarbophil with water or methanol (resulting concentration about 10-20 mg ml^{-1}) and the mixture is allowed to hydrate at room temperature for 24 hours. The polymer solution is periodically stirred. The resulting gel is applied on a cover glass and tested as described above and the result obtained is used as a reference value for excellent bioadhesive substances.

Similarly, other substances which are known bioadhesive substances are tested such as, e.g., chitosane, tragacanth, hydroxypropylmethylcellulose (HPMC), sodium alginate, hydroxypropylcellulose (HPC), karaya gum, carboxymethylcellulose (CMC), gelatin, pectin, acacia, PEG 6000, povidone, or DEAE-dextran (less bioadhesive than polycarbophil). By choosing test substances with various degrees of bioadhesiveness, an evaluation scale can be made and the performance of a test sample with respect to bioadhesiveness can be evaluated. It is contemplated that the following scale is applicable provided the test conditions given above are applied. It is clear that if the test conditions are changed, another scale may be more relevant. A suitable scale is then to be based on the values obtained for the excellent bioadhesive polycarbophil and the weak bioadhesive such as DEAE-dextran.

Bioadhesive properties	Work of adhesion (mJ cm^{-2})
none	less than 0.005
poor	about 0.005 - about 0.012
moderate	about 0.012 - about 0.020
good	about 0.020 - about 0.04
excellent	more than 0.04

When testing some known bioadhesive substances and GMO, the following results were obtained as a mean of six experiments:

Test substance	Work of adhesion (mJ cm^{-2})
DEAE-dextran	0.010
Sodium alginate	0.015
GMO 84/water 85/15% w/w*	0.028
HPMC	0.036
Carbopol 934	0.031
GMO 84	0.047
Polycarbophil	0.060

*: lamellar phase

3. In vivo test system for bioadhesion - washing off ability from the skin

A water soluble dye (Edicol Sunset Yellow, E 110, Amaranth E-123, or Brilliant Blue E 131) and/or a lipid soluble dye (Waxoline violet A FW (Maximex), Colur flavus insolubilis, DAK 63, or Edilake tartrazin NS) can be added to the test sample and mixed to form a homogeneous mixture. In those cases where a water soluble dye is used, the dye is preferably dissolved in an aqueous medium before mixing. In most cases, however, a dye is not added as the result is easily determined visually. About 0.05-0.5 g (such as 0.2 g) of the resulting mixture was applied in a uniform layer on an area of about 4 cm^2 of the skin of the hand or of the wrist. The test samples could be applied on dry skin as well as on moistened skin. In some cases, about 10 min before running the test, a small amount of water could be added to the test sample applied. Immediately after application, the test sample on the skin was subjected to washings with water from a tip (flow rate corresponding to about 6-8 litres/minute and a temperature of about 35-40°C). The washings were carried out for about 3 minutes. Then it is visually assessed in which degree the test sample is retained on the skin. The visual assessment is done by use of a scale graded from 1-5, where 5 represents total retainment of the test sampl applied on the skin and 1 represents no retainment of th test sample n the skin.

The test sample is evaluated to have bioadhesiv properties in the present context if the result of the above-described test is at least 4.

The test described above has proved to be suitable when testing compositions for bioadhesiveness and the compositions in question have a relatively high viscosity which makes it difficult to apply the compositions to the rabbit jejunum model. A modification of the test described above excluding the addition of a water soluble dye has also proved suitable for testing compositions for bioadhesiveness.

Quantitative determinations of glycerylmonooleate and glycerylmonolinoleate by means of HPLC

The quantitative determination of glycerylmonooleate or glycerylmonolinoleate was made by high-performance liquid chromatography (HPLC) using a Shimadzu LC-6A HPLC pump, a Shimadzu SPD-6A UV detector, a Shimadzu C-5A integrator and a Shimadzu SIL-6B autosampler.

The column (25 cm x 4 mm i.d.) was packed with Supelcosil LC-18-DM and was eluted isocratically at ambient temperature with a mobile phase consisting of methanol:water:acetate buffer (pH 3.5) (840:120:40 v/v). However, in some cases interference from other substances may occur, and then it may be necessary to make minor changes in the composition of the eluent.

The size of a sample injected on the column was 20 µl and the flow rate was 1.2 ml/ml. The column effluent was monitored at 214 nm.

Extraction procedure prior to analysis of glycerylmonooleate or glycerylmonolinoleate in mucosa

The mucosa in question (with a fatty acid ester, e.g. glycerylmonooleate) is placed in 50.00 ml of methanol and shaken for 2 hours. The mixture is filtered through a 0.45 µm filter membrane (from Millipore 16555Q) and the filtrate is subjected to HPLC analysis using the method described above.

Recovery

In those cases where analysis is performed in order to determine the residual amount of fatty acid ester (e.g. glycerylmonooleate) on the rabbit jejunum segment in connection with the bioadhesive test No. 1 (above), the calculation of the residual amount takes into consideration an appropriate correction in the recovery. This correction is found based on determination of the amount of fatty acid ester on the rabbit jejunum segment after application of an accurate amount of fatty acid ester (this test is repeated 5 times and the recovery is given as the mean value).

The recovery of about 125 mg GMO 84/ethanol 60/40% w/w on rabbit jejunum was examined. The recovery was found to be about 95%. The recovery was not determined for the other amounts of GMO/ethanol 60/40% w/w nor was it determined for GMO or GML formulations to which drug substances or excipients were added.

Solubility of acetylsalicylic acid (aspirin):

Wyatt D.M. and Dorschel O. A cubic phase delivery system composed of glyceryl, monooleate and water for sustained release of water-soluble drugs, Pharm. Tech. 1992 (Oct.), p. 116-130, disclose an experiment in which aspirin is used. Aspirin is not a substance which has a low solubility in water at a pH prevailing in the composition such as appears from the following.

Aqueous solubility

The solubility of the weak acid aspirin is 3.3 mg/ml in water (20°C). It has a pKa value of about 3.5 (25°C) (Analytical Profiles). The solubility of aspirin is strongly dependent on the pH in the solution. The degree of ionisation of the acid group in aspirin is favoured when the pH is around and above the pKa value of the compound and therefore the solubility is increased with pH > 3.4. A solubility experiment has shown that the solubility of aspirin is greater than 10 mg/ml in a buffer solution of pH 3.6. The experiment was performed in an 0.5 M acetate buffer solution pH 4.0; the buffer was not strong enough to maintain the pH, and the pH in the final solution was 3.6. The solubility of aspirin in a buffer solution of pH 4.0 is > 20 mg/ml.

Solubility in GMO/water

The solubility of acetylsalicylic acid in GMO/water 65/35% w/w has been determined to be >20 mg/ml. During the experiment, the pH of the aqueous phase at the end of the experiment was

4.0 and the aqueous phase used was 0.2 M acetate buff r pH 5.0 (the buffer used was not strong enough to maintain the pH at 5.0)

Determination of the dissolution/release rate of a pharmaceutical formulation

The dissolution rate of acyclovir in various GMO compositions was determined using Franz diffusion cells having a diffusion area of 1.77 cm^2 and a receptor volume of 6.8 ml. The study was run at a temperature of 37°C and as diffusion membrane a cellulose membrane from Medicell International Ltd. was employed. The membrane employed has a pore size of about 2.4 nm and it retains particles having a molecular weight larger than about 12,000-14,000. Before application, the membrane was pretreated and thoroughly rinsed with distilled water. As receptor medium was used an isotonic 0.05M phosphate buffer pH 6.5 (Danish Drug Standards, DLS) and the medium was magnetically stirred at 100 rpm.

The cellulose membrane was allowed to equilibrate at 37°C for 30 min in the receptor medium employed. After placing the membrane in the diffusion cell, about 300-350 mg of the composition to be tested was applied by means of a syringe or a spatula and care was taken to ensure a homogenous distribution of the composition on the total area of the membrane available for diffusion. Alternatively, the composition to be tested may be filled into a dish having a well-defined surface area which is only a little smaller than that of the cellulose membrane held by a Franz' diffusion cell so that almost all of the diffusion area available is used; the dish is turned upside down and placed on top of the cellulose membrane. Phosphate buffer was then loaded into the receptor part (time t=0) and at appropriate time intervals, samples of 2-3 ml were withdrawn and analyzed for content of acyclovir (cf. below). This relatively high volume was withdrawn to ensure sink condition. The amount of receptor medium withdrawn was replaced with fresh receptor medium.

Quantitative determination of miconazole and lidocaine hydrochloride, respectively

Samples from Example 12 were analyzed for the content of miconazol and lidocain hydrochloride, respectively. The following assays were employed:

Lidocain HCl

The content of lidocain HCl is determined by a HPLC method.

T: Dissolve the formulation in 30 ml methanol and transfer it quantitatively to a 50 ml volumetric flask. Add methanol to 50.00 ml.

R: Weigh out 100.00 mg lidocain HCl in a 100 ml volumetric flask. Dilute 1000 µl to 50.00 ml with mobile phase.

Analyse T and R on a suitable liquid chromatograph with UV-detector and integrator.

Column:	Steel column, length 25 cm x 4.6 mm i.d.
Stationary phase:	Nucleosil C-18, 10 µm
Mobile phase:	Methanol R: Acetic acid: Triethylamine: Water (50:1.5:0.5:48)
Flow:	1.5 ml/min
Temperature:	Room temperature
Detection:	254 nm
Injection:	20 µl loop
Retention time:	Lidocain HCl: about 3 min

Calculation:

$$\text{Lidocain HCL recovery, \%:} \frac{A_T \times n(g)}{A_R \times m(g) \times \%\ \text{lidocain HCl}} \times 100\%$$

where A_T is the area of the test solution T;
A_R is the area of the standard solution R;
n is the amount of standard weighed out (g);
m is the amount of formulation applied to the intestine (g);
% lidocain HCl is the content of lidocain HCl in the formulation determined as % w/w.

Miconazol

The content of miconazol is also determined by a HPLC method.

T: Dissolve the formulation in 30 ml methanol and transfer it quantitatively to a 50 ml volumetric flask. Add methanol to 50.00 ml.

R: Weigh out 100.00 mg miconazol in a 100 ml volumetric flask. Dilute 1000 µl to 50.00 ml with mobile phase.

Analyse T and R on a suitable liquid chromatograph with UV-detector and integrator.

Column:	Steel column, length 25 cm x 4.6 mm i.d.
Stationary phase:	Spherisorb ODS 1, S5
Mobile phase:	Methanol R: Buffer (85:15)
Flow:	1.0 ml/min
Temperature:	70°C
Detection:	230 nm
Injection:	20 µl loop
Retention time:	Miconazol: about 8 min
Buffer:	0.05 M $NH_4H_2PO_4$ (5.75 g in 1000 ml H_2O)

Calculation:

$$\text{Miconazol recovery, \%:} \frac{A_T \times n(g)}{A_R \times m(g) \times \% \text{ miconazol}} \times 100\%$$

where A_T is the area of the test solution T;

A_R is the area of the standard solution R;

n is the amount of standard weighed out (g);

m is the amount of formulation applied to the intestine (g);

% miconazol is the content of miconazol in the formulation determined as % w/w.

Quantitative determination of acyclovir

Method A

Determination of acyclovir in aqueous media by HPLC

The HPLC method employed was the following:

Column:	25 cm x 4.6 mm i.d.
Stationary phase:	Nucleosil C-18
Mobile phase:	water:methanol (85:15)
Temperature:	Room temperature
Detection:	254 nm
Flow:	1 ml/min
Inj.volume:	20 µl
Ret. time:	ca. 5.4 min

In connection with dissolution/release rate experiments employing Franz diffusion cells as described above, the concentration in the test solution (C_n) is calculated as follows:

Reference solution: An accurate amount of about 10.00 mg acyclovir is diluted to with distilled water to a concentration of 10.00 µg/ml

Test solution: The sample withdrawn is filtered through a 0.2 µm filter and injected onto the column (in some cases it might be necessary to subject the sample to dilution with water)

$$C_n = \frac{A_T \text{ x amount weight in (mg) of reference x } 1000 \text{ x } 5}{A_R \text{ x } 100 \text{ x } 50} \text{ µg/ml}$$

in which

A_T is the area of the test solution, and

A_R is the area of the reference solution.

Calculation of % released:

$$\frac{100 \text{ x } C_n \text{ x } V_t + (V_s \text{ x } \sum_{n=1}^{n} C_{n-1}))}{\text{\% of acyclovir in form. x mg of form. applied x } 1000} \text{ x } 100\%$$

in which

C_n is the concentration of drug in the receptor solution (mg/ml),

V_t is the receptor volume (unless otherwise stated, V_t = 6.8 ml),

V_s is the sample volume withdrawn,

C_{n-1} is the concentration in the previous sample (µg/ml).

Method B

Determination of acyclovir in pharmaceutical formulations by HPLC

The HPLC method employed was the following:

Column:	Steel column, 25 cm x 4.6 mm i.d.
Stationary phase:	Nucleosil C-18, 5 µm
Mobile phase:	water:methanol (20:80)
Temperature:	Room temperature
Detection:	254 nm

Flow:	0.8 ml/min
Inj.volume:	20 µl
Ret. time:	ca. 3.5 min

Reference solution: Weigh out an accurate amount of about 20.00 mg acyclovir and dilute it with mobile phase to a concentration of about 0.008 mg/ml

Test solution: Weight out 100.00 mg of the GMO/acyclovir formulation in a 50 ml volumetric flask. Dilute with mobile phase to 50.00 ml. Dilute 5.00 ml to 50.00 ml with mobile phase.

From the areas of the test solution and reference solution, respectively, the percentage of acyclovir present in the formulation is calculated.

Method C

Recovery of acyclovir on intestine

The HPLC method employed is the same as described under Method B. The test solution is prepared as follows:

The intestine is shaken for 2 hours with 50.00 ml of the mobile phase. The test solution is filtered through a 0.2 µm filter. Dilute 1000 µl to 10.00 ml with mobile phase.

From the areas of the test solution and reference solution, respectively, the percentage of acyclovir present in the formulation is calculated.

Determination of pH in the liquid crystalline phase

pH in the crystalline liquid phase is determined in a 10% w/w dispersion of the liquid crystalline phase (containing the active substance and any excipients) in distilled water. Prior to determination the dispersion is subjected to ultrasonic treatment for 30 minutes in order to ensure that an equilibrium between the liquid crystalline phase and the distilled water has taken place. The pH is measured by employment of a HAMILTON FLUSHTRODE which is a suitable pH-electrode for measurement of pH in the dispersions. The procedure followed was in accordance with the instructions given by the manufacturer of the electrode.

The method described above can be employed for various compositions, i.e. for composition wherein th concentration of the active ingredient in the liquid crystalline phase may be varied (e.g. from 1-20% w/w or in any range relevant for compositions according to the invention.

Modifications of the method described above may also be employed e.g. i) the dispersion mentioned above may obtained by diluting the liquid crystalline phase in a range corresponding to from about 1:20 to about 1:5 with distilled water, ii) ultrasonic treatment may be omitted or substituted by stirring provided that measures are taken to ensure that equilibrium takes place or, alternatively, that measurement of pH takes place after a well-defined time period, and iii) other suitable electrodes may be employed.

Most important is it to ensure that for comparative purposes the test conditions (stirring, ultrasonic treatment, time, electrodes) should be essentially the same when determining pH in the liquid crystalline phase of compositions.

In order to determine when an equilibrium between the liquid crystalline phase and the distilled water has taken place a number of experiments were performed varying the time period for ultrasonic treatment (0-5 hours) and measuring the pH immediate after the end of the ultra sonic treatment and 24 hours later. The experiments are performed on a GMO/water 65/35 containing 5% w/w of acyclovir. Based on the results of these experiments a time period of 30 minutes proved suitable, i.e. there is only an insignificant difference in the pH measured immediately after the end of ultra sonic treatment and 24 hours later.

Determination of drug solubility

The determination of the solubility of the active substance in the liquid crystalline phase of the composition is, of course, performed on the liquid crystalline phase as formed. In practice, this means that when the composition is one in which the liquid crystalline phase has already been formed when the composition is applied, the determination of the solubility is performed on the composition itself. The determination of the solubility is suitably performed by microscopy to observe any crystals of the active substance. Suitable test conditions involve a magnification of about 250 x and e.g. room temperature (20°C or 37°C may also be employed). The determination of the concentration at which crystals are observed is performed after a period of at least one week after preparation of the composition or the liquid crystalline phase to ensure that equilibrium has taken place. Normally, a series of tests with varying concentrations is performed to determine the concentration above which crystals are found. On the oth r hand, when the composition is a precursor composition, the liquid crystalline phase used as a reference in the solubility determination is a liquid crystalline phase imitating the liquid crystalline phase which

will be formed when the composition absorbs liquid from the site of application. This reference liquid crystalline phase is made up with water (as representing the liquid absorbed) in such an amount that the reference liquid crystalline phase is the same type of liquid crystalline phase as is generated from the precursor composition.

In order to determine the aqueous solubility of the active substance at the pH prevailing in the liquid crystalline phase, the pH is determined in the liquid crystalline phase as described above to determine the pH conditions when determining the solubility. [Many experiments with GMO have revealed that the pH of the liquid crystalline phase predominantly is about 4.5.] The solubility of the active substance is then determined by stirring an excess amount of the active substance in water, where applicable, being buffered to a pH substantially identical to the pH prevailing in the liquid crystalline phase for a time period of at least 24 hours (to ensure that equilibrium has taken place) and at a constant temperature (e.g. 20°C, room temperature or 37°C). In some case the samples initially were subjected to ultrasonic treatment for half an hour in order to accelerate the time for equilibrium. The concentration of the active substance in the supernatant (i.e. the aqueous solubility at the given pH) is then determined by an appropriate assay (e.g. by HPLC or UV spectroscopy).

As mentioned above, when the pH of the liquid crystalline phase, determined as described herein, is different from the pH which will result simply by dissolution of the active substance in water, the water is adjusted to substantially the pH of the liquid crystalline phase by using a suitable buffer system when determining the solubility of the active substance. This buffer system should of course be so selected that, apart from the pH adjustment, it has substantially no influence on the solubility of the active substance in the buffered water.

pH-solubility profile

Alternatively, the aqueous solubility is determined as a function of pH, i.e. by determining the aqueous solubility in buffer systems having a pH in a range of about 3 to about 9.5 such as about 3.6 to about 9. Suitable buffer systems include acetate, citrate, phosphate, borate etc. and the concentration of the buffer is sufficient to ensure a constant pH during the experiments. A concentration of at least 0.01 M is normally suitable. This method is applicable when determining the minimum aqueous solubility of a specific active substance at a given temperature and at a given pH range. The test conditions described (pH, temperature, ultrasonic treatment, stirring, time for ensuring that equilibrium has taken place) above are also valid when determining the minimum solubility.

Determination of liquid crystalline structure

Phase transitions of GMO 84 and/or GMO 90 containing compositions

In the following tests are described which make it possible to determine the crystalline structure of suitable compositions for use according to the invention. The tests allow determination of the presence of, e.g., the GMO 84 or GMO 90 in a lamellar, hexagonal or cubic phase, and it is possible to test the compositions before and after application to an appropriate application site. With respect to the various liquid crystalline phases formed by GMO or other glycerol fatty acid esters, an excellent review is given by Ericsson et al. in ACS Symp. Ser. (1991), pp 251-265, American Chemical Society and by Larsson in Chapter 8 (part 8.2.1 entitled "Lamellar and hexagonal liquid-crystalline phases") in The Lipids Handbook edited by Gunstone et al. In short, the lamellar phase is the dominating one at a relatively low water content (below 20% w/w) and at a temperature of about 37°C, whereas the cubic phase dominates as the water content increases (more than about 20% w/w).

A. Phase transition of GMO 84 and/or GMO 90 compositions determined by differential scanning calorimetry (DSC)

The DSC measurements were performed using a Perkin Elmer Unix DSC model 7 Differential Scanning Calorimeter. The heating rate was 5°C/min and the scanning temperature was from 5°C to 70°C. Samples were contained in sealed aluminium pans (Perkin Elmer No. BO14-3017) and as a reference empty aluminium pans were employed. The phase transitions caused only a relatively small enthalpy change and, therefore, the amount of sample tested was optimized to about 25 mg. The prepared pans were sealed and stored for two days at 5°C prior to analysis.

B. Phase transition of GMO 84 and/or GMO 90 compositions determined by polarimetry

The liquid crystalline phase can also be determined using polarized light and e.g. employing a stereomicroscope (Leitz, Diaplane) equipped with polarization filters. The appearance of reversed micelles (L2) are seen as a liquid oil, the lamellar phase (L_α) is mucous-like and in polarized light it is birefringent. The appearance of the cubic phase is as a very viscous and glass-clear sample. In polarized light the cubic phase (Q) is optically isotropic and gives a black background with no details indicating that it does not reflect the light. The lamellar and hexagonal phases are optically anisotropic. The lamellar phase gives a structure like a pipe cleaner on a black background or, expressed in another manner, could be identified from the oily streak texture and the spherical, positiv maltese cross-units visible between crossed polarisers. The reversed

hexagonal phase gives different patterns but in most cases it resembles a mosaic-like structure or gives angular or fan-like textures.

The method can be employed in testing the phase behaviour of various bioadhesive compositions.

C. Phase transition of GMO 84 and/or GMO 90 compositions determined by X-ray diffraction

A modified diffraction thermal pattern (DTP) camera was employed. The source was an X-ray tube equipped with a Cu-anode emitting Kα-rays at a wavelength of 1.5418 Å. The X-ray generator was a Philips PW 1729.

The liquid crystalline state can be identified by low angle X-ray diffraction and its appearance in polarized light. The characteristic X-ray diffraction pattern for the three liquid crystalline phases (lamellar, hexagonal, cubic) will give rise to diffraction lines in the following orders:

1:1/2:1/1:4...(lamellar)
1:1/√3:1/4:1/√7...(hexagonal)
1:1/√2:1/√3:1/√4:1/√5:1/√6:1/√8...(cubic)

In the case of the cubic form, the 3 different lattices will give rise to three different diffraction lines.

EXAMPLES

The following examples 1-11 relate to the preparation and structure of bioadhesive compositions or bioadhesive vehicles for use therein.

Unless otherwise stated, all percentages are by weight.

In all examples, the glycerylmonooleate (abbreviated as GMO in the following) (and whenever relevant glycerylmonolinoleate (Dimodan® LS)) is gently melted on a heating plate or in an oven and the liquid obtained (max. temperature of the melted liquid is about 60°C) is cooled to about 40°C before mixing with other ingredients. The monoglyceride mixtures and the ingredients wer mixed by stirring or shaking. In those cases where the composition contains an active substance in a GMO/ethanol or GML/ethanol vehicle, one of the following methods can be applied:

1. the active substance was dissolved or dispersed in ethanol and then mixed with melted GMO under stirring,
2. the active substance was dissolved or dispersed in melted GMO and then ethanol was added under stirring,
3. the active substance was dissolved or dispersed in a GMO/ethanol mixture.

When storing at room temperature (22°C) some formulations become inhomogeneous. In relevant cases the formulations were melted and stirred to obtain a homogeneous mixture before use.

The acyclovir ointment composition was prepared as follows:

In general, the acyclovir was suspended in the melted GMO and the other ingredients were added. The monoglyceride mixtures and the ingredients were mixed by stirring or shaking. In the case of compositions containing TPGS, the acyclovir was added to the TPGS solution before mixing with GMO. The compositions were subjected to ultrasound treatment for about 1 h and were stored for at least two days at 37°C before use to ensure that equilibrium had been obtained (e.g. that the stable liquid crystalline phase has been formed in the total formulation and that equilibrium between the solid and dissolved substance has taken place). As an alternative to adding the acyclovir to the melted GMO, the acyclovir can be suspended in the liquid phase before combining the liquid phase with the melted GMO.

In those cases where a bioadhesive test is performed, the values given are mean values of the results of 2-4 tests. It should be noted that the values given in the Examples are not corrected for recovery, i.e. the values are minimum values. If a correction for recovery is made the values will become larger.

The test conditions for performing Test No. 1 for bioadhesiveness are:

angle:	-21°
initial rinsing period:	5 minutes
initial rinsing flow:	10 ml/min
flow rate:	10 ml/min
flow period:	30 minutes

EXAMPLE 1

Preparation of a semi-solid (c l urless gel) compositi n without any active substance

The composition was prepared from the following ingredients:

GMO	65 g
Water	35 g

The GMO and water were mixed by shaking. The liquid crystal structure of the gel obtained is cubic as evidenced by polarized light.

The composition was tested for bioadhesiveness in test system No. 3 (washing off ability). A score of 4-5 was found indicating that the composition is bioadhesive.

EXAMPLE 2

Preparation of a semi-solid (grey white) acyclovir ointment composition

GMO	61.8 g
Water	33.3 g
Acyclovir (crystalline)	5.0 g

The liquid crystal structure of the gel obtained is cubic as evidenced by both polarized light and X-ray diffraction (see below).

The composition was tested for bioadhesiveness in test system No. 3 (washing off ability). A score of 4-5 was found indicating that the composition is bioadhesive. A similar result was obtained by employment of test system No. 2 (tensiometry).

EXAMPLE 3

Preparation of a semi-solid (milk white) acyclovir ointment composition

GMO	61.8 g
Water	33.3 g
Acyclovir (micronized)	5.0 g

The liquid crystal structure of the gel obtained is cubic as evidenced by both polarized light and X-ray diffraction (see b low).

The composition was tested for bioadhesiveness in test system No. 3 (washing off ability). A score of 4-5 was found indicating that the composition is bioadhesive.

Preliminary tensiometric measurements (test system No. 2) confirmed that compositions containing 5% w/w crystalline acylovir were bioadhesive. The compositions tested were GMO 90 with 5% w/w acyclovir and GMO 90/water 65/35% w/w with 5% w/w acyclovir.

EXAMPLE 4

Preparation of a semi-solid composition without an active substance

The composition was prepared from the following ingredients:

GMO	85 g
Water	15 g

The GMO and water were mixed by shaking and a lamellar phase of GMO was obtained as evidenced by polarized light.

The composition was tested for bioadhesiveness in test system No. 1. A residual amount of about 84% w/w GMO was found after testing.

A composition of GMO/water 90/10% w/w was prepared in the same manner and gave a residual amount of about 87% after testing in test system No. 1.

The compositions were also tested for bioadhesiveness in test system No. 3 (washing off ability). A score of 4 was found indicating that the compositions are bioadhesive.

EXAMPLE 5

Preparation of a semi-solid composition (colourless gel) without an active substance

The composition was prepared from the following ingredients:

GMO	65 g
Glycerol	35 g

The GMO and glycerol were mixed by shaking.

The liquid crystal structure of the gel obtained is cubic as evidenced by polarized light.

The composition was tested for bioadhesiveness in test system No. 3 (washing off ability). A score of 4-5 was found indicating that the composition is bioadhesive.

EXAMPLE 6

Preparation of a liquid composition without an active substance

The composition was prepared from the following ingredients:

GMO	50 g
Ethanol	30 g
Glycerol	20 g

The GMO was mixed with ethanol and glycerol was added to the resulting mixture while stirring.

The composition was tested for bioadhesiveness in test system No. 1. A residual amount of about 81% w/w GMO was found after testing.

EXAMPLE 7

Preparation of a liquid composition without an active substance

The composition was prepared from the following ingredients:

GMO	60 g
Ethanol	30 g
Benzyl alcohol	10 g

The GMO was mixed with ethanol, and benzyl alcohol was added to the resulting mixture while stirring.

The composition was tested for bioadhesiveness in test system No. 1. A residual amount of about 87% w/w GMO was found after testing.

EXAMPLE 8

Preparation of a semi-solid composition without an active substance

The composition was prepared from the following ingredients:

Dimodan® LS	65 g
Water	35 g

Water was added to the Dimodan® LS under vigorous stirring.

The composition was tested for bioadhesiveness in test system No. 3 (washing off ability). A score of 4-5 was found indicating that the composition is bioadhesive.

EXAMPLE 9

Preparation of a sprayable composition without an active substance

The composition was prepared from the following ingredients:

Dimodan® LS	60 g
Ethanol	40 g

Ethanol was added to Dimodan® LS and mixed.

The composition was tested for bioadhesiveness in test system No. 1. A residual amount of about 95% w/w GMO was found after testing.

EXAMPLE 10

Acyclovir containing compositions

In the following table is listed a number of acyclovir containing compositions according to the invention. The compositions were prepared as described above. 5% w/w acyclovir was added to all the compositions listed in the table below.

Composition	%w/w
GMO 90/water	98/2
GMO 90/water	95/5
GMO 90/water	90/10
GMO 90/water	85/15
GMO 90/water	80/20
GMO 90/water	75/25
GMO 90/water	72/28
GMO 90/water	71/29
GMO 90/water	70/30
GMO 90/water	69/31
GMO 90/water	68/32
GMO 90/water	67/33*
GMO 90/water	66/34
GMO 90/water	65/35
GMO 90/water	64/36
GMO 90/water	63/37
GMO 90/water	62/38
GMO 90/water	61/39
GMO 90/water	60/40
GMO 90/water	55/45

* To this composition of GMO/water 10%, 20% and 30% w/w acyclovir, respectively, was added

The compositions having from about 55 to about 80% w/w GMO (based on the content of GMO and water) are cubic at room temperature.

The compositions having from about 95 to about 98% w/w GMO (based on the content of GMO and water) are probably the reversed micellar phase (L_2) (precursor of the cubic phase).

The compositions having from about 80 to about 90% w/w GMO (based on the content of GMO and water) are the lamellar phase (L_α) (precursor of the cubic phase).

The presently most promising compositions are those having a content of from about 55 to about 80% such as, e.g., from about 60 to about 75%, from about 65 to about 70% w/w GMO (based on the content of GMO and water).

Compositions containing glycerol and/or lecithin were also prepared as described above. 5% w/w acyclovir was added to all the compositions listed in the table below. All compositions are cubic.

Composition	%w/w
GMO 90/water/glycerol	60/20/20
GMO 90/water/glycerol	65/20/15
GMO 90/water/glycerol	65/25/10
GMO 90/water/glycerol	67/20/13
GMO 90/water/glycerol	70/10/20
GMO 90/water/glycerol	70/15/15
GMO 90/water/glycerol	70/20/10
GMO 90/water/glycerol	75/15/10
GMO 90/water/lecithin	55/35/10

All the listed cubic compositions (both the compositions based on GMO 90/water, GMO 90/water/glycerol and GMO 90/water/lecithin, respectively) were bioadhesive (evidenced by employment of test system No. 3). A score of 4-5 was generally obtained. Furthermore, the cubic phase of all the compositions are stable at 25°C (60% relative humidity) and at 40°C (75% relative humidity). Under the test conditions acyclovir has been found to be stable in the cubic phase and, furthermore, GMO has also proved to be stable. The stability mentioned above is valid for at least 1 year (the term "stability" in the present context is generally understood by a person skilled within the pharmaceutical field as denoting that a decrease in content of a specific substance of at the most 10% based on the initial value has been observed).

EXAMPLE 11

Compositions containing antiviral substances

In the following table is listed a number of interesting compositions. The compositions are prepared as described above. 5% w/w of an antiviral substance is add d to all the compositions listed in the table below.

Vehicle	Composition % w/w
GML/water	65/35 + 5% acyclovir
GMO 90/water	67/33 + 5% penciclovir
GMO 90/water	67/33 + 5% famciclovir
GMO 90/water	67/33 + 5% ganciclovir
GMO 90/water	67/33 + 5% valaciclovir
GMO 90/water	65/35 + 10% acyclovir
GMO 90/water	65/35 + 10% cidofovir
GMO 90/water	65/35 + 10% lobucavir
GMO 90/water	65/35 + 10% sorivudine
GMO 90/water	65/35 + 20% didanosine

Other compositions are also relevant, i.e. compositions having other active substances or having a drug concentration of about 1-10% w/w and compositions having a composition of the vehicle as given in Example 10 above.

Example 11

pH-solubility profile for acyclovir

Experimental

To a 100 ml Erlenmeyer flask were added 50 ml buffer solution and 250 mg acyclovir.

The buffers with pH 3.6, 4.2 and 5.3 were prepared using monobasic sodium phosphate and dibasic sodium phosphate (pH adjustment with phosphoric acid). The buffers in the pH range 6.0 to 9.6 were prepared using monobasic potassium phosphate (pH adjustment with dodium hydroxide). The molarity of the phosphate salts was 0.05M; the pH of the medium was measured with a pH-meter.

Each mixture was stirred with a magnetic stirrer for 24 hours, and after equilibrium to room temperature, the sample was passed through a membrane filter. The solution was diluted to appropriate volume and the amount of acyclovir dissolved was determined by HPLC.

The solubility of acyclovir as a function of pH is given in the table below and in Fig. 3. From the r sults, it is seen that the minimum solubility of acyclovir is at a pH in a range of from about 4 to about 6.

Acyclovir/Solubility at different pH

pH (buffer)	Acyclovir, mg/ml
3.6	1.9
4.2	1.8
5.3	1.8
6.0	1.8
6.6	1.9
7.6	1.9
8.5	2.2
8.8	2.5
9.0	2.5
9.2	2.9
9.6	3.5

EXAMPLE 13

Investigation of the influence of different active substances on the liquid crystalline phase

Miconazole is an example of an active substance which is insoluble in water but has a solubility of more than 2% w/w in the liquid crystalline phase. However, the release of miconazole is very slowly from the cubic phase. The table given below shows the solubility of and the crystalline phase obtained for miconazole in a GMO/water 70/30% w/w vehicle.

Miconazole (% w/w)	Solubility	Liquid crystalline phase
1	soluble	cubic
2	soluble	cubic
3	soluble	cubic
4	soluble	cubic
5	soluble	cubic
6	crystals	lamellar

For miconazole (as well as for some other substances which are soluble in the cubic liquid crystalline phase in certain concentrations) experiments have shown that the bioadhesiveness of

compositions containing the substances varies with the concentration of the substance. In the table below results are given from testing various miconazole compositions in a GMO/ethanol 60/40% w/w vehicle or in a GML/ethanol 60/40% w/w vehicle, respectively, for bioadhesiveness employing Test system No. 1.

Concentration of miconazol (% w/w)	Bioadhesion* (residual amount %)	
	GMO-based	GML-based
0	85	95
2		
3		
4	72	86
5		41
6	72	
8	33	
10		4
15		
25		

*: In the tests runs the following test conditions were employed: initial rinsing period: 5 min, initial rinsing flow: 10 ml/min, angle: -21°, flow rate: 10 ml/min, flow period: 30 min

From the results given above for the GMO-based composition it is seen that there is a dramatic fall in bioadhesiveness when the concentration of miconazole exceeds 6% w/w, i.e. when the liquid crystalline phase changes from the cubic phase to the lamellar phase and when miconazole in the liquid crystalline phase is present as crystals, i.e. when the concentration exceeds the solubility of miconazole.

The results support the results of other experiments performed by the inventors, namely that there is a close correlation between the presence of a cubic phase and occurrence of a high degree of bioadhesiveness. The other experiments performed by the inventors involved application of GMO, GMO/ethanol mixtures, GML on Test system No. 1 for bioadhesiveness. It was found that the samples applied in contact with th mucosa and washing medium all had converted into the cubic phase and that the samples were bioadhesive. The same applies for

comp sitions containing indomethacin (5% w/w) in a GMO/ethanol 60/40% w/w vehicle and other bioadhesive compositions containing an active substance.

From the results given above in the table it is seen that when the concentration of miconazol exceeds a certain level, the bioadhesion is severely impaired. This indicates that when the concentration of the active substance in the cubic phase exceeds a certain level, the cubic phase structure is disturbed, or another liquid crystalline phase may perhaps have been formed (the active substance and/or any excipients may alter the phase diagram).

In the case of acyclovir, however, this reduction in bioadhesiveness with increased content of acyclovir, beyond the saturation point, does not seem to influence the cubic phase and does not seem to impair the bioadhesiveness (tested by means of Test system No. 3). Experiments showing this were performed with acyclovir ointment compositions, prepared with GMO 90, with concentrations of crystalline acyclovir of 2%, 5%, 10%, 20% and 30% by weight, respectively. These compositions were found to be highly bioadhesive, indicating that with substances having a very low solubility in the liquid crystalline phase, the liquid crystalline phase remains less disturbed by the presence of particles of the active substance and retains its bioadhesive properties.

EXAMPLE 14

Investigation of the influence of different excipients or solvents on the bioadhesiveness of GMO or GML based compositions

The influence of various excipients and solvents was investigated. The various compositions were prepared as described above and the bioadhesiveness was tested using the test system No. 1. The following results were obtained:

Composition % w/w	Bioadhesion Residual amount %
GMO[a]	90
GML[a]	65*
GMO/GML[a] 40/60***	56*
Mixtures with solvents:	
GMO/water 85/15[b]	94

GML/ethanol 60/40	95**
GMO/ethanol/propylene glycol/water:	
45/30/10/15	93
Mixtures with solubilizing	
agents or preservatives:	
GMO/ethanol/benzyl alcohol:	
60/30/10	87**
GMO/ethanol/benzyl alcohol/water:	
60/20/5/15	80
50/20/10/20	89
Mixtures with release modulating	
agents:	
GMO/ethanol/glycerol:	
50/30/20	97
GMO/ethanol/sesame oil:	
59/40/1	96
58/40/2	93
50/40/10	14
50/30/20	0**
GMO/ethanol/soybean oil:	
59/40/1	98
58/40/2	93
50/40/10	22
40/20/40	0**
GMO/ethanol/lecithin:	
55/40/5	99
45/40/15	97

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[a] melted gently before application

[b] lamellar phase

* lower results than expected; probably due to the reference values used in the analysis of the mixture

** test conditions as in Examples 1-12

*** the GMO/GML mixture corresponds to about equal amounts of glycerol monooleate and glycerol monolinoleate

The results given above show that addition of relevant excipients or solvents such as, e.g., agents which are known solubilizers for active substances or agents which are known as release modulating agents (i.e. agents which when added make it possible to adjust or control the release of the active substance from a composition) do not significantly influence the bioadhesiveness of the composition when the agents (excipients or solvents) are added in relatively low concentrations (less than about 10% w/w). Thus, the release of an active substance from a composition which has proved to possess bioadhesive properties can be controlled at least to a limited extent by adjusting the amount of a release modulating agent such as, e.g., glycerol, sesame oil, soybean oil, sunflower oil, lecithin, cholesterol, etc. A modulating agent may influence the pore size of the water channels in the cubic phase and/or alter the partition coeffient of the active substance between the cubic phase and the aqueous phase at least to a limited extent. Furthermore, if necessary, solubilisation of an active substance or a fatty acid ester for use in a bioadhesive composition can be effected by use of e.g. benzyl alcohol without significantly influencing the bioadhesive properties of the composition. In conclusion, the bioadhesive principles described herein have a high potential with respect to developing bioadhesive drug compositions having such a drug localization, such a drug release profile, and such a drug duration which are desirable or necessary under the given circumstances. Thus, the present inventors have found an advantageous bioadhesive drug delivery system.

EXAMPLE 15

Investigation of the presence of an active substance in a liquid crystalline phase of glycerol monooleate

The methodology described herein is a methodology which is generally useful for investigating whether mixing or dissolving of an active substance in a vehicle capable of forming a liquid crystalline phase also leads to incorporation of the active substance in the liquid crystalline phase. While miconazol and lidocain hydrochlorides have been used as model substances in the description of the experiments, the same measures as described herein can be used for substances which have a very low solubility in both water and ethanol such as, e.g., acyclovir.

Furthermore, the study was performed in order to examine the recovery of the samples applied.

A lipophilic (miconazol) and a hydrophilic active substance (lidocain hydrochloride), respectively, were applied on the rabbit jejunum test model for bioadhesiveness (test system No. 1). A vehicle of GMO 84/ethanol 60/40% w/w incorporating 2% w/w of either miconaz l or lidocain hydrochloride was employed. The GMO 84/ethanol vehicle is bioadhesiv in itself. After a flow

period of 10 sec (corresponding to t=0), and a flow period of 30 minutes (corresponding to the end of the experiment) the samples appli d were removed from the mucosa and th cubic phase was quantitatively examined by HPLC for the content of active substance. As seen from the table below almost all miconazole was found after 10 sec and 30 minutes. These results indicate that the lipophilic miconazole is incorporated in the cubic phase formed and the result at 30 minutes indicates that the drug is very slowly released from the cubic phase. This is consistent with release experiments of miconazole delivered from a cubic phase into a 0.05 M phosphate buffer solution, pH 6.5 (37°C). Miconazole seems to prefer the lipophilic part of the cubic phase. The results are given in the following table; results for an acyclovir composition are also given.

Composition	Flow period	Recovery of active substance % mean of two determinations
GMO 84/ethanol/miconazol:		
58.8/39.2/2	10 sec	85
	30 min	93
GMO 84/ethanol/lidocain HCl:		
58.8/39.2/2	10 sec	37
	30 min	7
GMO 90/acyclovir		
95/5	10 sec	87
	30 min	65

In the experiment with lidocaine hydrochloride, barely half the content of the drug was recovered after a flow period of 10 sec and only a negligible amount after 30 minutes. Because of its high water solubility (about 0.7 g/ml at 25°C), the greater part of the lidocaine hydrochloride is probably dissolved and washed away in the buffer solution during the prehydration time (10 min) and only some is incorporated in the cubic phase formed. Most of the incorporated drug had been released at the end of the experiment. Other studies have shown that lidocaine hydrochloride is released rather quickly from the cubic phase probably through the water channels contained in the cubic phase.

Results for acyclovir, which is poorly soluble in both water and the cubic phase, given in the tabl clearly demonstrate that acyclovir is enclosed in the cubic liquid crystalline phase formed and some of it may have been released during the experiment.

In conclusion, the experiments reported above indicate that formulations in which GMO and an active substance are dissolved in ethanol or the active substance suspended in GMO 90, serve as a precursor for the formation of a cubic phase formed in situ, and that the active substance is incorporated in the cubic phase formed.

EXAMPLE 16

Phase transitions of GMO 84 containing compositions

A. Compositions without any drug substance

The composition of Example 4, i.e. a composition of GMO 84/water 85/15% w/w, is tested employing the DSC method described under the heading "Methods" above. The results are given in Fig. 4. DSC experiments give information about at which temperature a phase conversion takes place. DSC measurements alone give no information of the particular phases involved (e.g. lamellar, cubic hexagonal etc.). However, if the DSC results as in the present case are compared with e.g. results from observation of the compositions in polarized light (see above under the heading "Methods") information on the crystalline phases as well as the transition temperature is obtained.

For the composition from example 4, the results from the DSC and polarized light measurement show that the lamellar phase is present at room temperature and the lamellar phase is changed to the cubic phase when the temperature increases (Fig. 4). The transition temperature is about 37°C.

B. Compositions containing acyclovir

DSC experiments as described above were also performed on compositions containing GMO/water 65/35% w/w with 5% w/w acyclovir (crystalline (Example 2) and micronized (Example 3), respectively). The samples were stored at 5°C for two days to ensure equilibration of the sample. The lipids in th sample solidified at this temp rature. The DSC was run at 5-70°C. The thermograms obtained showed only a clear melting peak at about 16-17 °C for both the reference sample (GMO/water 65/35% w/w) and the samples containing 5% w/w acyclovir. The solidified sample transfers to the cubic phase (reversible process). No phase transition of the

cubic phase seemed to have taken place. The results are in well agreement with the results obtained by use of X-ray diffraction measurement described in the following.

Compositions containing GMO/water 65/35% w/w and GMO/water 65/35% w/w with acyclovir (crystalline and micronized, respectively) added in concentrations 2.5, 5.0 and 10% w/w were subjected to X-ray diffraction measurements (as described under the heading "Methods") in a temperature scan at 20-70°C. The aim of the study was to examine if the cubic phase of GMO/water 65/35% w/w is changed when acyclovir is added. In the following results from the compositions of Example 2 and 3, respective, at 37°C are given for illustrative purposes:

d-Spacings:

	Ex. 2	Ex. 3	Ratio
Lipid phase	61.7Å	61.7Å	1
	50.5Å	50.5Å	0.81
	36.3Å	36.3Å	0.58
	29.7Å	29.7Å	0.48
Acyclovir	12.9Å	-	-
	8.44Å	-	-
	3.74Å	3.74Å	-
	3.42Å	3.42Å	-

The results show that the compositions are cubic at 37°C.

The results obtained for all the tested compositions in the temperature range 20-70°C show that all the tested compositions are cubic in the temperature interval 20-70°C. The diffraction lines from acyclovir do not interfere with the lines from the cubic phase. In conclusion, the results indicate that acyclovir both in its crystalline and micronized form is inert in the cubic phase. Thus, no influence of acyclovir on the phase behaviour has been observed in the concentration range investigated and the cubic phase containing acyclovir is rather stable against temperature fluctuations.

Furthermore, compositions containing GMO/water 65/35% w/w with acyclovir (crystalline and micronized, respectively) added in a concentration of 1-40% were tested in polarized light at 22°C and 37°C, respectively, as described above under the heading "Methods". The results show the presence of cubic phases in all compositions indicating that acyclovir probably is inert in the cubic phase.

EXAMPLE 17

Dissolution/release rate of a bioadhesive composition containing acyclovir

The dissolution rate of acyclovir in various GMO compositions was determined using Franz diffusion cells as described under the heading "Methods".

A series of GMO compositions containing acyclovir were prepared as described above, and they were subjected to the above dissolution rate determination. All compositions were suspensions of acyclovir, that is, they contain acyclovir which was not dissolved. The solubility of acyclovir in the compositions investigated was less than 0.1% w/w (0.05% w/w<the solubility of acyclovir<0.1% w/w).

The results appear from Figures 5-11. The results indicate that the release of acyclovir from a GMO based vehicle is dependent on the concentration of acyclovir in the composition, provided that the release takes place from a cubic phase system. Furthermore, the results indicate the capability of a GMO-based vehicle to function as a very effective drug delivery system.

Figs. 5-7 show the release of acyclovir (1-5% micronized) from a cubic phase (GMO/water 65/35% w/w) and Zovir® cream, respectively, into isotonic 0.05 M phosphate buffer solution, pH 6.5 (37°C). As appears from the graph of Fig. 5 showing the cumulative release of acyclovir, the release of acyclovir increases with increasing concentration of acyclovir over the range investigated. There is not proportionality between the rate of release and the concentration; this appears from the fact that the graphs of % released (Fig. 6) do not coincide and the slope of the Higuchi plots (Fig. 7); the release is dependent on the concentration.

It is justified to refer to rate constant herein as the release of acyclovir from the liquid crystalline formulations according to the invention which can be described by means of the so-called Higuchi equation (Higuchi, T., Rate of release of medicaments from ointment base containing drug in suspension. J. Pharm. Sci., 50 (1961) 874-875): on linear regression, the cumulative amount of acyclovir released plotted versus the square root of time results in a straight line with the slope k (rate constant $\mu g/h^{1/2}$). This appears from Fig. 7 which shows the plots for a number of compositions containing acyclovir in concentrations from 0.99% by weight to 4.76% by weight in comparison with Zovir® cream containing 5% by weight of acyclovir. The slopes of the graphs in Fig. 7 are as follows:

Zovir® cream, 5%:	155
Acyclovir 0.99% w/w:	410

Acyclovir 1.96% w/w:	587
Acyclovir 2.91% w/w:	717
Acyclovir 3.85% w/w:	773
Acyclovir 4.76% w/w:	1016

The higher the acyclovir concentration is, the smaller the percentage of acyclovir released. This would indicate that acyclovir must first be dissolved before it is released from the cubic phase, probably through the water channels. In other words, the rate of dissolution seems to be a rate limiting factor, to the rate of release in the diffusion process. In spite of this, and in spite of the low solubility of acyclovir both in water and in the cubic phase, the release of acyclovir from the composition according to the invention is dramatically increased compared to the Zovir® cream. Thus, a comparison of the rate constant for acyclovir (5%) released from Zovir® cream and GMO/water 65/35% w/w shows that the rate constant is about 6 times larger for the latter (Fig. 7).

With a view to testing if the rate of release can be improved by means of micronized acyclovir, as opposed to crystallinic acyclovir, the release from various compositions was examined. Figures 8, 9 and 10 show an identical release pattern for crystalline and micronized acyclovir, respectively, from a formulation consisting of GMO/water 65/35% w/w + 1% acyclovir. On the other hand it appears that the release rate of crystalline acyclovir is slightly improved from a composition containing lecithin (GMO/water/lecithin 55/35/10% w/w + 1% acyclovir) compared to the same composition containing micronized acyclovir (Figs. 8-9). By comparing the release profiles for compositions consisting of GMO/water 65/35% w/w containing 5% crystalline and 5% micronized acyclovir, respectively (Fig. 10), it seems that the release rate has increased somewhat with the micronized quality. On the other hand other studies have indicated that the release rate of acyclovir from a composition consisting of GMO/water/glycerol % w/w + 5% acyclovir is identical for the crystallinic and the micronized quality. Whether the release is improved by application of a micronized quality as opposed to a crystalline quality depends on the composition of the cubic phase. However, more experiments have to be carried out to exclude that the differences observed arise from experimental variation.

The micronized quality increases the viscosity of the cubic phase more that the crystalline phase. This condition alone favours the use of the crystalline quality in a potential product so that product of suitable and not too high viscosity can be obtained. Furthermore, the use of the crystalline form is favourable from a stability point of view.

The release of acyclovir from various GMO compositions containing 1% w/w and 5% micronized acyclovir, respectively, containing release modulating or solubilising compounds was examin d

and compared with the release from a cubic phase consisting of 65 parts of GMO and 35 parts of water (figs. 9-10). All the compositions except the compositions containing sesame oil and the composition containing GMO/glycerol 65/35 % w/w were the cubic phase, as evidenced in polarised light. As can be seen from the release profiles in Fig. 11 for the compositions containing 1% acyclovir, the profile of GMO/water 65/35 % w/w (reference) has a shape similar to the others with the exception of the profiles for the compositions containing sesame oil. In the latter case the release speed is drastically reduced, which could mean that the compositions consist of the reversed hexagonal phase, but this has not been confirmed. It should be noted that the composition consisting of 65 parts of GMO and 35 parts of glycerol, have the same release profile as the reference composition, although both the visual and the polarized light do not indicate that they consist of the cubic phase. It is possible however, that the cubic phase is created on the surface of the formulation during the release experiment, through its contact with the dissolution medium (37°C). Addition of the release modulating substances glycerol and lecithin to the cubic phase has not significantly changed the release of acyclovir in the concentrations examined. Neither does the TPGS seem to have increased the dissolution of acyclovir in the cubic phase nor changed the partitions' coefficient between the cubic phase and the release medium, as the release profile is identical with the profile of the reference composition. Fig. 11 shows the release profiles of composition containing 5% acyclovir. The release profiles for the compositions containing glycerol and lecithin are identical while the release profile of the reference composition is somewhat smaller. This indicates that the release of acyclovir is slightly increased from the compositions added release modulation agents, however, the improvement is modest. The tests indicate that it is difficult to change the release of acyclovir significantly. There are limited possibilities for changing the release if the cubic structure is to be preserved.

EXAMPLE 18

Case stories on treatment of cold sores - preclinical study in humans

A composition of GMO/water 65/35% w/w with 5% w/w acyclovir has been used for the treatment of cold sores in humans.

Treatment was started with a maximum of 24 hours delay from start of symptoms. In one case, treatment with Zovirax® cream was tried for 4.5 days before switch to GMO acyclovir cream. GMO acyclovir cream was applied 3 times daily (range 2-4) f r 2.5 days (range 1.5-4).

The results of the study are given in the Table below.

Case	Demographics	Daily applications	Treatment duration (days)	Outcome	Side effects
1	38 year ♀	3	2	Symptoms ceased. Superficial ulceration for one week	Erythema
2	50 year ♂	3	2,5	Symptoms ceased	None
3	52 year ♀	2	2,5	Symptoms ceased	None
4	29 year ♀	4	4	Symptoms improved	None
5a	38 year ♀	2	4	Ulceration	Treatment stopped due to dry skin
5b	38 year ♀	2	3	Symptoms ceased	Treatment stopped. Ulceration healed in 2 weeks. Erythema 2 weeks
6	35 year ♂	3	1,5	Healing	None
7	30-40 year ♂	No information	2-3	7/8 improved 1/8 no effect	None
Summary		Median 3 (2-4)	Median 2,5 (1,5-4)		5/7 None 2/7 erythema 1/7 ulceration 1/7 dry skin

In 7 of 8 treatments symptoms ceased or improved very much. In one case, ulceration occurred and treatment was stopped. Healing was only reported in one case, probably because the treatment prevented the typical ulceration of a cold sore.

Side effects were noted by 2 to 7 persons. One of these persons received two treatments and in both cases, treatment was stopped due to side effects. The side effects reported were ulceration, transient erythema and dry skin. No severe or serious side effects were reported.

The reported case stories do not represent scientific evidence of the efficacy of GMO acyclovir cream. They do, however, indicate that the characteristics of GMO/water 65/35% w/w with 5% acyclovir on certain points differ from those of Zovirax® cream from Glaxo Wellcome. GMO/water 65/35% w/w with 5% acyclovir adheres firmly to skin. Therefore fewer daily applications of GMO/water 65/35% w/w with 5% acyclovir were administered than what is recommended for Zovirax® cream. In 6 out of 8 cases, treatment could successfully be stopped after 2-3 days. This is shorter than the normally recommended treatment period for Zovirax® cream of 5 to 10 days.

Application frequency and treatment duration for GMO acyclovir cream in these case reports are less than recommended for Zovirax® cream. By the persons treated, the efficacy was judged to be equivalent or better than that of Zovirax® cream.

EXAMPLE 19

Skin irritation of GMO/water 65/35% w/w with 5% acyclovir

The Chamber Scarification Test has been used in order to evaluate the skin irritation profile of GMO/water 65/35% w/w with 5% acyclovir.

The Chamber Scarification Test is developed to investigate and compare cosmetics, cosmetic ingredients and consumer products intended for repeated use on normal or diseased skin. The assay amplifies irritant reactions to the test products by scarification of the test area prior to the first application.

The implications of results of a Chamber Scarification Test using 20 volunteers (ProDERM study # 94,011-05) with GMO 70% in water showing a mean sum of score of about 8 are evaluated with respect to the potential for causing unacceptable skin irritation.

An average score around 8 is in th high range and comparable to the irritation caused by known products such as Chlorhexidine ointment 1% and the old formulation of Helosan ointment. These products may be used for a short period of time without causing subjective or visible skin reactions.

A high score in a Chamber Scarification Test is problematic for products intended for daily use over a long period, on sensitive skin areas and in individuals with hyperirritable skin.

Recurrent herpes simplex infections are a nuisance for the patients because of itching, oozing, paraesthesia and skin eruptions lasting from several days to a few weeks. If an improved acyclovir cream clears the eruption in a few days, it may be regarded as a very good therapeutic effect compared to the spontaneous course of the disease, irrespective of a certain degree of skin irritation that may be caused by the topical drug. This possible irritation may not be detected at all due to the herpes symptoms.

The new cream is meant to be applied on the herpes simplex infected skin area without occlusion twice daily for a few days. In the vast majority of patients, it will probably be tolerated without any problems. After the herpes attack, no further applications of the drug will be performed until the next herpes attack months later.

The product has several favourable characteristics. i.a. increased bioadhesiveness and increased bioavailability for acyclovir. Furthermore, pilot experiments in volunteers with recurrent herpes simplex have shown that the product is well tolerated and leads to rapid clearing of the herpes attack.

EXAMPLE 20

In vitro permeability of compositions according to the invention across porcine skin

Test substances

1. GMO/Water 65/35% w/w added 5% w/w micronized acyclovir BFJ30-1
2. GMO/Water 65/35% w/w added 5% w/w crystalline acyclovir BFJ30-3
3. Zovir® 5%, Wellcome (containing 5% acyclovir) BFJ15-6

Preparation of skin membranes

Excised abdominal skin from pigs was obtained from University of Copenhagen, The Panum Institute, Department of Experimental Surgery. The hairs were removed from the epidermal side by clipping. Subcutaneous fat on the dermal side was removed. The skin was washed with distilled water and stored at -18°C until use.

Apparatus

Franz diffusion cells having an available diffusion area of 1.77 cm^2 were used. The epidermal side of the skin was exposed to ambient laboratory conditions while the dermal side was bathed with the receptor medium consisting of 6.8 ml of 0.05 M phosphate buffer, pH 6.5. Each cell was placed on a magnetic stirrer. The temperature of the water flowing in the closed circulatory system was kept at 37°C.

Permeation procedure

The skin membranes were thawed and mounted in Franz diffusion cells. The receptor chambers were filled with receptor medium and the epidermal side of the skin was wetted with a few drops of receptor medium. The skin was then allowed to equilibrate for about 24 hours. Blood and soluble enzymes were at the same time washed out of the skin, and thereby could not disturb analysis of the receptor medium for acyclovir. The integrity of the individual skin samples was ensured by measuring the capacitance of the skin. Skin samples with a capacitance of less than about 0.055 μF were considered intact, whereas skin samples with a higher capacitance were considered damaged. The water permeability may also be determined as a measure of the integrity of the skin. Before application of the test substances, the receptor medium was replaced by fresh media. 300-350 mg of the test substance was spread across the entire epidermal surface in an even layer. At appropriate intervals (t=0, 6, 24, 30, 48 hours) 2 ml samples were withdrawn and replaced by fresh receptor medium keeping an infinite sink. Due to variation when using biological membranes, at least eight permeation studies were performed on each test substance.

Permeation tests over pig skin have shown that acyclovir from Zovir® cream and the cubic phase permeates more or less at the same rate during the first 48-hours periods (see Fig. 12).

However, release tests in vitro have shown that acyclovir incorporated into a cubic phase of GMO (GMO/water 65/35% w/w + 5% acyclovir) is released approx. 5-6 times faster than acyclovir from Zovir® cream.

EXAMPLE 21

In vitro permeability of compositions according to the invention across human skin

A. Wholly skin

In order to evaluate the influence of the compositions on the ability of acyclovir or other antiviral compounds to penetrate the stratum corneum and to accumulate in the epidermis and the dermis, the following experiments can be performed using wholly intact human skin excised from cosmetic surgery. The skin is obtained from clinics for plastic surgery. The skin is treated as mentioned in the Example above and stored at -18°C. Skin from other mammals than humans may also be employed such as, e.g. guinea pigs, mice and pigs. The skin may be separated into epidermis and dermis by exposing the skin to hot water (60°C) for e.g. 30 seconds (heat separation) or by slicing with a microtome (mechanical separation). The stratum corneum can be isolated by tape stripping. The test conditions are generally as described in the Example above, but other test times (e.g. from 1 hour to 7 days), amounts of sample applied (e.g. 50-350 mg) etc. may be appropriate. To avoid intra-individual variations the same donor is used to testing different compositions and the skin specimens were taken from the same skin area. In order to simulate injured skin, the skin can be injured by applying a skin enhance or by stripping the skin with tape.

The amount of drug substance within the skin can be calculated by measuring the concentration of the drug substance in i) the receptor medium, ii) the skin, and/or iii) the remaining composition. By measuring i) and iii), the amount of drug substance in the skin can be calculated.

B. Different layers of the skin

The herpes virus replicate in the living epidermis. The basal layer of the epidermis appears to be the primarily site of antiviral activity in cutaneous HSV-1 infections, i.e. the epidermis appears to be the target site for antiviral drug substances.

Permeation (i.e. penetration into and through the skin) of acyclovir or other antiviral substances can be investigated across isolated epidermis by diffusion (as described above). In this manner, a measure is obtained of the amount of acyclovir having permeated the epidermis. Alternatively, a picture is obtained of the penetration (i.e. the entry into the skin but not through the skin) of acyclovir (or other antiviral substances) in the skin by means of diffusion test using wholly skin

which at the end of the experiment is divided into stratum corneum, epidermis and dermis by means of a microtome. The individual layers are analysed for acyclovir (or other antiviral substances), e.g. by liquid scintillation.

In those cases where radioactive acyclovir (or other radioactive antiviral drugs) are used, the amount of acyclovir penetrating the tissue was measured by a liquid scintillation technique (^{3}H-acyclovir is commercially available in form of a ethanol/water 30/70 solution). In order to examine the content of acyclovir in different skin sections/layers, the skin sections were placed in scintillation vials with e.g. Soluene 350 over night to dissolve the skin components. Scintillation cocktail was subsequently added and the samples were assayed for content of acyclovir (or the appropriate antiviral drug) by liquid scintillation spectrometry. The drug metabolizing enzyme activity in the epidermis is greatly dependent on tissue viability. Therefore, it should be stressed that the determination of skin absorption described above does not distinguish between the intact antiviral drug and its metabolites. It cannot be excluded that excised skin (usually stored) will loose some of its original enzyme activity. However, acyclovir exhibits no known metabolism in the skin.

By extracting acyclovir from the skin components, acyclovir can also be quantified by HPLC.

EXAMPLE 22

Permeation of compositions containing acyclovir or other drugs by means of an in vitro cell culture model

The permeation of acyclovir or other antiviral drugs delivered from various compositions according to the invention can be examined using in vitro cell cultures as a model of e.g. human oral epithelium. A model involving e.g. TR 146 cell (from the Royal Danish School of Pharmacy, Copenhagen, Denmark) is suitable for sensitivity and permeability studies of antiviral drugs. Other cell culture models are also available, e.g. for the testing of the efficacy of drugs.

EXAMPLE 23

Permeation of compositions containing acyclovir or other antiviral drugs by means of an in vivo animal model

The herpes virus replicate in the living epidermis. The basal layer of the pidermis appears t be the primary site of antiviral activity in cutaneous HSV-1 infections, i.e. the target for antiviral drugs. Methods - using hairless mouse as an animal model - are available. The methods allow

calculation of the target site concentration of the antiviral (e.g. acyclovir) drug applied and allow an estimation of the efficacy of the antiviral compositions tested (see. e.g. Lee, P.H. et al., Pharm. Res. 9, 8, pp 979-988, 1992 and Su, M.-H. et al., Drug Develop. Ind. Pharm. 20 (4), 685-718, 1994). In the following is described model systems suitable for testing the antiviral effect of the compositions according to the invention.

Animal models often used are the hairless mouse model (5-7 weeks old) and the guinea pig model. The guinea pigs are shaved on their back before the start of the experiment in order to make a hairless test area.

The animals are anaesthetized before inducing skin lesions, e.g. on the lateral side of the body or in the lumbosacral area. 0.005-0.2 ml of a virus suspension [herpes simplex virus type 1 (HSV1), e.g. strain E-377 or E-115 (titer usually in a range of 10^6 - 10^8 plaque forming units (PFU)/ml), stored at -70°C until use] was injected or rubbed on the skin with a cotton swab saturated with the virus (a drop of the virus suspension is applied on the test area and then 6 small holes are made by means of a scalpel. The test area on the skin of the test animal can be divided into several test areas, e.g. six areas, thereby allowing e.g. two different compositions (2x2) and their controls (1x2), placebo) to be tested at the same time on the same animal. Usually 10-30 animals are used for each composition (the number of animals depends on the number of applications). 1 day prior to (and also after) inoculation the area can advantageously be treated with an enhancer such as, e.g., Azone, ethanol, sodium laurylsulfate or propylene glycol. The infection induced by the virus generated skin lesions which appeared at the area of inoculation. Shortly after virus inoculation (e.g. 24 hours) compositions with antiviral drugs were applied on the test areas at the skin e.g. with a 1 ml syringe and samples are blindly randomized (if desirable, pretreated with an enhancer like Azone). The lesions are treated with the compositions for 2-10 days (appied 2-5 times daily) and then the effect of the treatment was investigated. The lesions were scored for each animal and two distinct antiviral assessments can be made: i) topical efficacy is determined by measuring the antiviral activity of the antiviral drug substance (e.g. acyclovir) delivered from the compositions tested, and ii) systemic efficacy is determined by measuring the antiviral activity of the antiviral drug substance (e.g. acyclovir) in the circulatory system which delivers the antiviral substance to the target site (presumably the epidermal basal layer).

In order to quantify the effect of the different compositions, a score system is used. Different score systems may be employed based on the appearance of the skin lesions at various times after inoculation. The score system could be that of Alenius and Öberg, Archives of Virology 1978, 58, 277-288, where the course of infection is divided into a phase of progression denoted by scores with Arabic numerals and into a phase of regression denoted by scores with Roman

numerals. E.g. the inoculated areas can be scored for symptoms daily, starting 24 hours after inoculati n and ending after 4-20 days, giving scores during the development of vesicles and their subsequent drying and crusting. The length and size of skin lesions can also be measured. A low cumulative score of a composition indicates a good efficacy compared to a placebo composition (control) which generally gives a high score.

During the test HSV-1 virus may be isolated from the lesions and the number is counted. The results give an indication of i) inactivation of virus, ii) effect of the antiviral composition applied etc.

EXAMPLE 24

Clinical development programme of GMO acyclovir cream for herpes labialis

The following parameters are suggested for all clinical studies:

Setting

Outpatients from GPs, dermatologists or hospital clinics. Primary recruitment possible in connection with a Herpes simplex eruption that are not included in the study. Patients receive study medication and are instructed to start treatment immediately upon recurrence of prodromes and to return to investigator after start of treatment.

Inclusion criteria

Clinically confirmed history of recurrent Herpes Labialis, 2-3 annual recurrences. Present prodromal symptoms of Herpes Labialis eruption.

Exclusion criteria

Herpes labialis with ulceration or crusts
Immunodeficiency
Allergy to acyclovir/GMO

Efficacy parameters

Duration days/hours from start of tr atment to cessation of symptoms caused by virus replication, including pain, weal, numbness and erythema.

Duration days/hours form start of treatment to crust formation.
Duration days from start of treatment to complete skin healing.

Safety parameters
Local reactions to cream administration, including a 28-30 day follow-up.

Dose finding

The experience from individual case reports indicates that fewer daily applications of GMO acyclovir compared to Zovirax® are required to obtain efficacy. The optimal administration frequency will have to be determined.

Study groups:
Placebo
Once daily
Twice daily
Three times daily

If more than three daily applications is required, GMO acyclovir is not considered to have any advantage compared to Zovirax® cream.

At present no data are available on the statistical variation of efficacy parameters, therefore a proper dimensioning of the study has not been possible. It is assumed that between 100 and 200 patients per study group is required.

Pivotal studies

It is assumed that two identical or at least very similar studies must be performed.

Study groups
Placebo
GMO acyclovir x times daily
Zovirax® 5 times daily

The argument for including a placebo group in the pivotal study is to document that the expected clinical equivalence between Zovirax® and GMO acyclovir is not a consequence of both products inefficiency.

At present no data are available on the statistical variation of efficacy parameters, therefore a proper dimensioning of the studies has not been possible. It is assumed that between 100 and 200 patients per study group is required.

CLAIMS

1. A pharmaceutical composition for administration of an active substance to or through a nail or a damaged or undamaged skin or mucosal surface of an animal such as a human, the composition comprising the active substance and an effective amount of a fatty acid ester which, together with a liquid phase, is capable of generating a liquid crystalline phase in which the constituents of the composition are enclosed,

the composition either being one in which the liquid crystalline phase has been generated by the fatty acid ester together with a sufficient amount of a liquid phase originally present in the composition, or the composition being in a precursor form in which fatty acid ester has not generated the liquid crystalline phase, but is capable of forming the liquid crystalline phase in situ with moisture from the surface on which the composition is applied, the moisture in this case constituting at least part of the liquid phase

the pH of the liquid crystalline phase being in the range of 3.6-9, determined as described herein,

the active substance having

a solubility in the liquid crystalline phase of at the most 20 mg/g at 20°C, and

a solubility in water of at the most 10 mg/ml at 20°C, the water, where applicable, being buffered to a pH substantially identical to the pH prevailing in the liquid crystalline phase, determined as described herein,

with the proviso, where applicable, that the composition is not one consisting of either 2% by weight of acyclovir and 98% by weight of a glycerylmonooleate or 5% by weight of acyclovir and 95% by weight of a glycerylmonooleate product, wherein the glycerylmonooleate product has the composition:

Glycerylmonooleate	80-85% w/w
Glycerylmonolinoleate	5-10% w/w
Saturated monoglycerides	6-10% w/w.

2. A pharmaceutical composition for administration of an active substance to or through a nail or a damaged or undamaged skin or mucosal surface of an animal such as a human, the composition comprising the active substance and an effective amount of a fatty acid ester which,

together with a liquid phase, is capable of generating a liquid crystalline phase in which the constituents of the composition are enclosed,

the composition either being one in which the liquid crystalline phase has been generated by the fatty acid ester together with a sufficient amount of a liquid phase originally present in the composition, or the composition being in a precursor form in which fatty acid ester has not generated the liquid crystalline phase, but is capable of forming the liquid crystalline phase in situ with moisture from the surface on which the composition is applied, the moisture in this case constituting at least part of the liquid phase

the pH of the liquid crystalline phase being in the range of 3.6-9, determined as described herein,

the active substance having

a solubility in the liquid crystalline phase of at the most 20 mg/g at 20°C, and

a minimum aqueous solubility of at the most 10 mg/ml at 20°C determined at a pH in the range of 3.6-9, determined as described herein,

with the proviso, where applicable, that the composition is not one consisting of either 2% by weight of acyclovir and 98% by weight of a glycerylmonooleate or 5% by weight of acyclovir and 95% by weight of a glycerylmonooleate product, wherein the glycerylmonooleate product has the composition:

Glycerylmonooleate	80-85% w/w
Glycerylmonolinoleate	5-10% w/w
Saturated monoglycerides	6-10% w/w.

3. A composition according to claim 1 or 2, which contains at least 20% by weight, calculated on the composition, of the fatty acid ester.

4. A composition according to claim 3, which contains at least 30% by weight, preferably at least 40% by weight, calculated on the composition, of the fatty acid ester.

5. A composition according to any of the preceding claims, in which the liquid crystalline phase has a pH in the range of 3.6-8, determined as described herein.

6. A composition according to claim 5, in which the liquid crystalline phas has a pH in the range of 3.7-8, determined as described herein.

7. A composition to claim 5, in which the liquid crystalline phase has a pH in the range of 3.8-8, such as 3.9-8, e.g. 4.0-8, such as 4.1-8, eg. 4.2-8, e.g. 4.3-8, such as 4.5-8, e.g. 4.75-8, such as 5.0-8.

8. A composition according to any of the preceding claims, in which the solubility of the active substance in water is at the most 7 mg/g at 20°C, such as e.g. at the most 5 mg/g, and at a pH substantially identical to the pH of the liquid crystalline phase, determined as described herein.

9. A composition according to any of claims 1-7, in which the minimum aqueous solubility of the active substance is at the most 7 mg/g such as, e.g., at the most 5 mg/g at 20°C determined at a pH in the range of 3.6-9, determined as described herein.

10. A composition according to claim 8, in which the solubility of the active substance in water is at the most 3 mg/g such as, e.g., at the most 2 mg/g at 20°C and at a pH substantially identical to the pH of the liquid crystalline phase, determined as described herein.

11. A composition according to claim 9, in which the minimum aqueous solubility of the active substance is at the most 3 mg/g such as, e.g., at the most 2 mg/g at 20°C determined in a pH range of 3.6-9, determined as described herein.

12. A composition according to any of the preceding claims, in which the solubility of the active substance in the liquid crystalline phase is at the most 15 mg/g at 20°C.

13. A composition according to claim 12, in which the solubility of the active substance in the liquid crystalline phase is at the most 10 mg/g such as, e.g., at the most 7 mg/g or at the most 6.5 mg/g at 20°C.

14. A composition according to claim 13, in which the solubility of the active substance in the liquid crystalline phase is at the most 6 mg/g such as, e.g., at the most 5.5 mg/g or at the most 5 mg/g at 20°C.

15. A composition according to claim 14, in which the solubility of the active substance in the liquid crystalline phase is at the most 4 mg/g at 20°C, such as at the most 3 mg/g, at the most 2 mg/g r at the most 1 mg/g at 20°C.

16. A composition according to any of the preceding claims, in which the active substance is present in a concentration above the saturation concentration at 20°C.

17. A composition according to claim 16, wherein the proportion of the active substance present which is above the saturation concentration at 20°C is at least 25% by weight of the active substance present in the composition.

18. A composition according to claim 17, wherein the proportion of the active substance present which is above the saturation concentration at 20°C is at least 50% by weight of the active substance present in the composition.

19. A composition according to claim 18, wherein the proportion of the active substance present which is above the saturation concentration at 20°C is at least 75% by weight of the active substance present in the composition.

20. A composition according to claim 19, wherein the proportion of the active substance present which is above the saturation concentration at 20°C is at least 90% by weight of the active substance present in the composition.

21. A composition according to claim 20, wherein the proportion of the active substance present which is above the saturation concentration at 20°C is at least 95% such as at least 98% by weight of the active substance present in the composition.

22. A composition according to any of the preceding claims, in which the liquid crystalline phase is a cubic phase.

23. A composition according to any of the preceding claims, wherein the fatty acid moiety or moieties of the fatty acid ester is/are saturated or unsaturated and each have a carbon atom number from C_6 to C_{26}.

24. A composition according to claim 23, wherein the fatty acid moiety or moieties is/are a moiety or moieties of a saturated fatty acid selected from the group consisting of caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, and behenic acid.

25. A composition according to claim 23, wherein the fatty acid moiety or moieties of the fatty acid component is/are unsaturated.

26. A compositi n according to claim 25, wherein the fatty acid moiety or moieties is/are selected from the group consisting of palmitoleic acid, oleic acid, linoleic acid, linolenic acid, and arachidonic acid.

27. A composition according to any of the preceding claims, wherein the fatty acid ester is selected from the group consisting of fatty acid esters, in particular partial fatty acid esters, of polyhydric alcohols, fatty acid esters of hydroxycarboxylic acids, fatty acid esters of monosaccharides, fatty acid esters of glycerylphosphate derivatives, fatty acid esters of glycerylsulfate derivatives, and mixtures thereof.

28. A composition according to claim 27, wherein the polyhydric alcohol is selected from the group consisting of glycerol, 1,2-propanediol, 1,3-propanediol, diacylgalactosylglycerol, diacyldigalactosylglycerol, erythritol, xylitol, adonitol, arabitol, mannitol, and sorbitol.

29. A composition according to claim 28, wherein the fatty acid ester is selected from the group consisting of glycerylmonooleate, glycerylmonolinoleate, glycerylmonolinolenate, and mixtures thereof.

30. A composition according to claim 27, wherein the hydroxycarboxylic acid is selected from the group consisting of malic acid, tartaric acid, citric acid, and lactic acid.

31. A composition according to claim 27, wherein the fatty acid ester is a fatty acid monoester of citric acid.

32. A composition according to claim 27, wherein the monosaccharide is selected from the group consisting of glucose, mannose, fructose, threose, gulose, arabinose, ribose, erythrose, xylose, galactose, sorbose, altrose, tallose, idose, rhamnose, and allose.

33. A composition according to claim 32, wherein the fatty acid ester is a fatty acid monoester of a monosaccharide selected from the group consisting of sorbose, galactose, ribose, and rhamnose.

34. A composition according to claim 27, wherein the glycerylphosphate derivative is a phospholipid selected from the group consisting of phosphatidic acid, phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositole, and diphosphatidylglycerol.

35. A composition according to claim 27, wherein the fatty acid ester is a fatty acid ester of a glycerylph sphate derivativ or a glycerylsulfate derivative, and the fatty acid component is

selected from the group consisting of lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, lin l ic acid, linolenic acid, and behenic acid.

36. A composition according to claim 35, wherein the fatty acid ester is selected from the group consisting of dioleyol phosphatidylcholin, dilauryl phosphatidylcholin, dimyristyl phosphatidylcholin, dipalmitoyl phosphatidylcholin, distearoyl phosphatidylcholin, dibehenoyl phosphatidylcholin, dimyristyl phosphatidylethanolamine, dipalmitoyl phosphatidylethanolamine, dioleyl phosphatidylglycerol, dilauryl phosphatidylglycerol, dimyristoyl phosphatidylglycerol, dipalmitoyl phosphatidylglycerol, distearoyl phosphatidylglycerol, dipalmitoyl phosphatic acid and mixtures thereof.

37. A composition according to claim 27, wherein the fatty acid ester is glycerylmonooleate or glycerylmonolinoleate

38. A composition according to claim 37, wherein the fatty acid ester is glycerylmonooleate.

39. A composition according to claim 38, wherein the glycerylmonooleate product contained in the composition contains at the most 4% of saturated monoglyceride.

40. A composition according to claim 37, wherein the glycerolmonooleate product contained in the composition contains at least 88% such as at least 89% of glycerylmonooleate.

41. A composition according to claim 40, wherein the glycerolmonooleate product contained in the composition contains at least 90%, such as at least 91%, in particular at least 92%, of glycerylmonooleate.

42. A composition according to any of the preceding claims, wherein the liquid phase is present in an amount of at least 0.5% by weight, such as at least 1% by weight, calculated on the total composition.

43. A composition according to any of the preceding claims, wherein the liquid phase is present in an amount of at least 2% such as at least 5% by weight, calculated on the total composition.

44. A composition according to any of the preceding claims, wherein the liquid phase is present in an amount of at least 10% by weight, calculated on the total composition.

45. A composition according to any of the preceding claims, wherein the liquid phase is present in an amount of at least 20% by weight, calculated on the total composition.

46. A composition according to any of the preceding claims, wherein the liquid phase is present in an amount of at least 25% such as at least 30% by weight, calculated on the total composition.

47. A composition according to any of the preceding claims, wherein the liquid phase is present in an amount of 25-50% such as 30-50% by weight, calculated on the total composition.

48. A composition according to any of the preceding claims, wherein the liquid phase is present in an amount of 27-40% such as 30-40% or 27-37% by weight, calculated on the total composition.

49. A composition according to any of the preceding claims, wherein the active substance has a lipophilicity of at the most 100 such as at the most about 75, 50, 40, 30, 25, 10, 7.5, 5 or 2.5, expressed as the partition coefficient between octanol and 0.05M phosphate buffer, pH 7.

50. A composition according to claim 49, wherein the partition coefficient is at the most 1 such as at the most about 0.75, 0.5, 0.1, 0.075.

51. A composition according to claim 49, wherein the partition coefficient is at the most 0.05 such as at the most about 0.04.

52. A composition according to any of claims 1-48, wherein the active substance has a lipophilicity of at the most 100 such as at the most about 75, 50, 25, 10, 7.5, 5 or 2.5, expressed as the partition coefficient between octanol and an appropriate buffer having a pH corresponding either to the pH of the liquid crystalline phase or to the pH at which the active substance has its minimum solubility.

53. A composition according to claim 52, wherein the partition coefficient is at the most 1 such as at the most about 0.75, 0.5, 0.1, 0.075.

54. A composition according to claim 52, wherein the partition coefficient is at the most 0.05 such as at the most about 0.04.

55. A composition according to any of the preceding claims, wherein the release of the active substance from the liquid crystalline phase, as defined by the slope of the cumulative release in μg as a function of the square root of the release time in hours in the release experiment defined in Example 17 (in which the concentration of the substance is 5%), is at least 50.

56. A composition according to claim 55, wherein the slope is at least 100.

57. A composition according to claim 56, wherein the slope is at least 200.

58. A composition according to claim 57, wherein the slope is at least 300.

59. A composition according to claim 58, wherein the slope is at least 500.

60. A composition according to claim 59, wherein the slope is at least 700.

61. A composition according to claim 60, wherein the slope is at least 900.

62. A composition according to any of the preceding claims, in which the fatty acid ester or combination of fatty acid esters present in the composition complies with the requirements of bioadhesion defined herein when tested for bioadhesion in an in vivo model.

63. A composition according to any of the preceding claims, which complies with the requirements of bioadhesion defined herein when tested for bioadhesion in an in vivo model.

64. A composition according to claim 62, in which the fatty acid ester or combination of fatty acid esters, when tested in a bioadhesive test system, comprising

i) placing a segment of longitudinally cut rabbit jejunum on a stainless steel support in such a manner that the mucosa layer of the jejunum is placed upside so as to allow application of said fatty acid ester,

ii) placing the resulting support at an angle of -21° ± 2° in a cylindrical cell thermostated at 37°C ± 0.5°C and with the relative humidity kept at about 100%,

iii) flushing the jejunum on the support with 0.02M isotonic phosphate buffer solution (pH 6.5, 37°C) for 5 min at a flow rate of 10 ml/min,

iv) applying an accurately weighed amount of a sample of said fatty acid ester (about 100 mg) on a surface area (about 0.8 x 6 cm) of the mucosa of the jejunum on the support,

v) dropping about 0.5 ml of said phosphate buffer solution on the sample applied,

vi) leaving the resulting sample from step v) for 10 minutes in said cell to allow the sample to interact with glycoproteins of th jejunum,

vii) flushing the jejunum with the sample applied with said phosphate buffer solution (pH 6.5, 37°C) for 30 minutes at a flow rate of 10 ml/min,

viii) collecting the washings resulting from step vii), and

ix) calculating the residual amount of the sample remaining on the jejunum by measuring the amount of the sample in the washings or by measuring the amount remaining on the jejunum,

results in a residual amount of at least 60% w/w.

65. A composition according to claim 62, wherein the residual amount is at least 70% w/w.

66. A composition according to claim 65, wherein the residual amount is at least 80% w/w.

67. A composition according to claim 66, wherein the residual amount is at least 85% w/w.

68. A composition according to claim 67, wherein the residual amount is at least 90% w/w.

69. A composition according to any of claims 62-64 which complies with the requirements for bioadhesion defined herein when tested for bioadhesion in the in vivo model described herein involving testing the rinsing off ability from skin.

70. A composition according to any of claims 62-64 which, when tested in the test system defined in claim 64, results in a residual amount of at least 40% w/w of the fatty acid ester or combination of fatty acid esters or at least 40% w/w of the active substance.

71. A composition according to any of the preceding claims, wherein the active substance is an antiviral drug.

72. A composition according to claim 71, wherein the antiviral substance is selected from nucleosides, phosphorylated nucleosides, nucleoside analogues, nucleotide analogues, and salts, complexes and prodrugs thereof.

73. A composition according to claim 72, wherein the antiviral substance is selected from acyclovir, famciclovir, deciclovir, penciclovir, zidovudin, ganciclovir, didanosin, zalcitabin, valaciclovir, sorivudine, lobucavir, brivudine, cidofovir, n-docosanol and ISIS-2922.

74. A composition according to claim 73, wherein the antiviral substance is acyclovir.

75. A composition according to claim 74, wherein the fatty acid ester of the composition is in the form of the liquid crystalline phase generated together with the liquid phase.

76. A composition according to claim 74 or 75, wherein the fatty acid ester is a glycerylmonooleate product having a glycerylmonooleate content of at least 88% such as, e.g., at least about 89 or 90% by weight and a content of saturated monoglycerides of at the most 4% by weight.

77. A composition according to claim 76, wherein the content of glycerylmonooleate in the glycerylmonooleate product is at least 91% by weight.

78. A composition according to claim 76, wherein the content of glycerylmonooleate in the glycerylmonooleate product is at least 92% by weight.

79. A composition according to claim 78, wherein the content of saturated monoglycerides in the glycerylmonooleate product is at the most 2% by weight.

80. A composition according to claim 79, wherein the weight ratio between the glycerylmonooleate and the liquid is in the range between 50:50 and 75:25.

81. A composition according to claim 80, wherein the weight ratio between the glycerylmonooleate and the liquid is in the range between 63:37 and 73:27 such as between 60:40 and 70:30.

82. A composition according to claim 72, in which the fatty acid ester is not present in the form of a liquid crystalline phase, but is capable of forming the liquid crystalline phase in situ with moisture from a surface on which the composition is applied.

83. A composition according to claim 82, wherein the weight ratio between the glycerylmonooleate and any liquid is between 80:20 and 100:0.

84. A composition according to claim 83, wherein the weight ratio between the glycerylmonooleate and any liquid is between 90:10 and 99:0.5, such as between 90:10 and 99:1.

85. A composition according to any of claims 75-84, wherein the liquid is water or glycerol, or a mixture of water and glycerol.

86. A composition according to claim 85, wherein the liquid is water.

87. A c mposition according to claim 86, wherein the liquid is water containing glycerol in an amount of up to corresponding to a glycerol:water ratio of 2.5:1 by weight, such as up to corresponding to a glycerol:water ratio of 1.5:2 such as, e.g., a ratio of about 1:1, 0.5:1, or 0.25:1.

88. A composition according to any of the preceding claims comprising glycerylmonooleate, lecithin and, optionally, water and the weight ratio between the content of lecithin and glycerylmonooleate is at the most 1, such as e.g. 1:1, 1:2 or 1:4.

89. A composition according to claim 88, wherein the concentration of water in the composition is at the most 40% w/w based on the total composition.

90. A composition according to any of the preceding claims further comprising glycerol.

91. A composition according to claim 90, wherein the total concentration of glycerol and any water present is at the most 40% w/w based on the total composition.

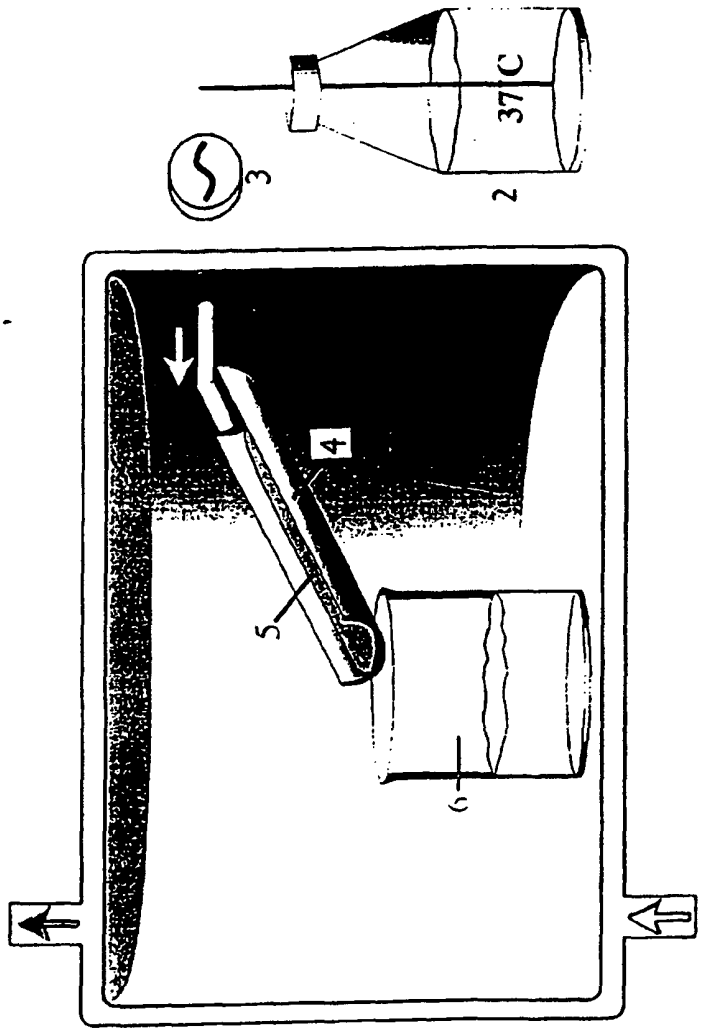

Fig. 1

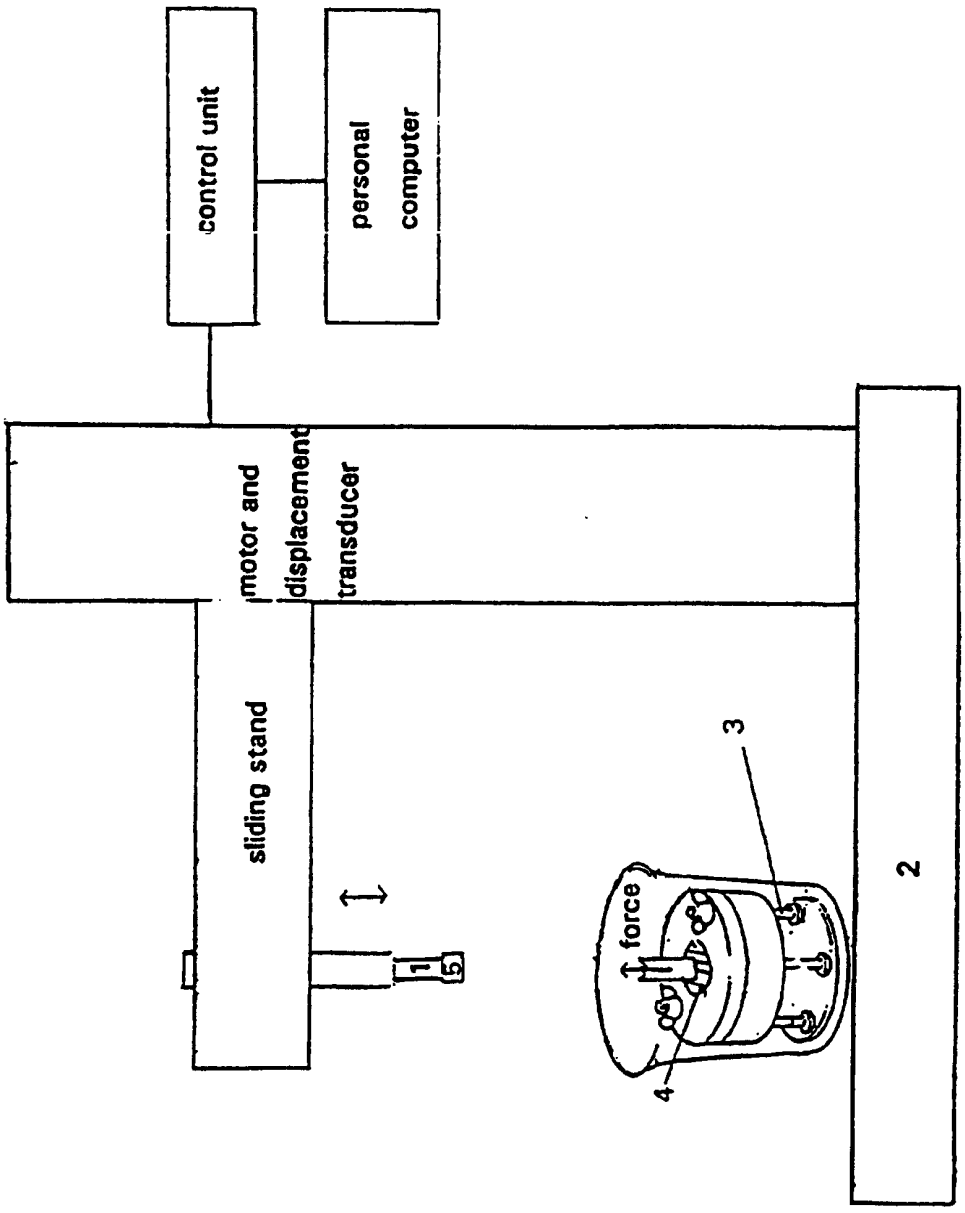
control unit
personal computer
motor and displacement transducer
sliding stand
force
1
2
3
4
5

Fig. 2*a*

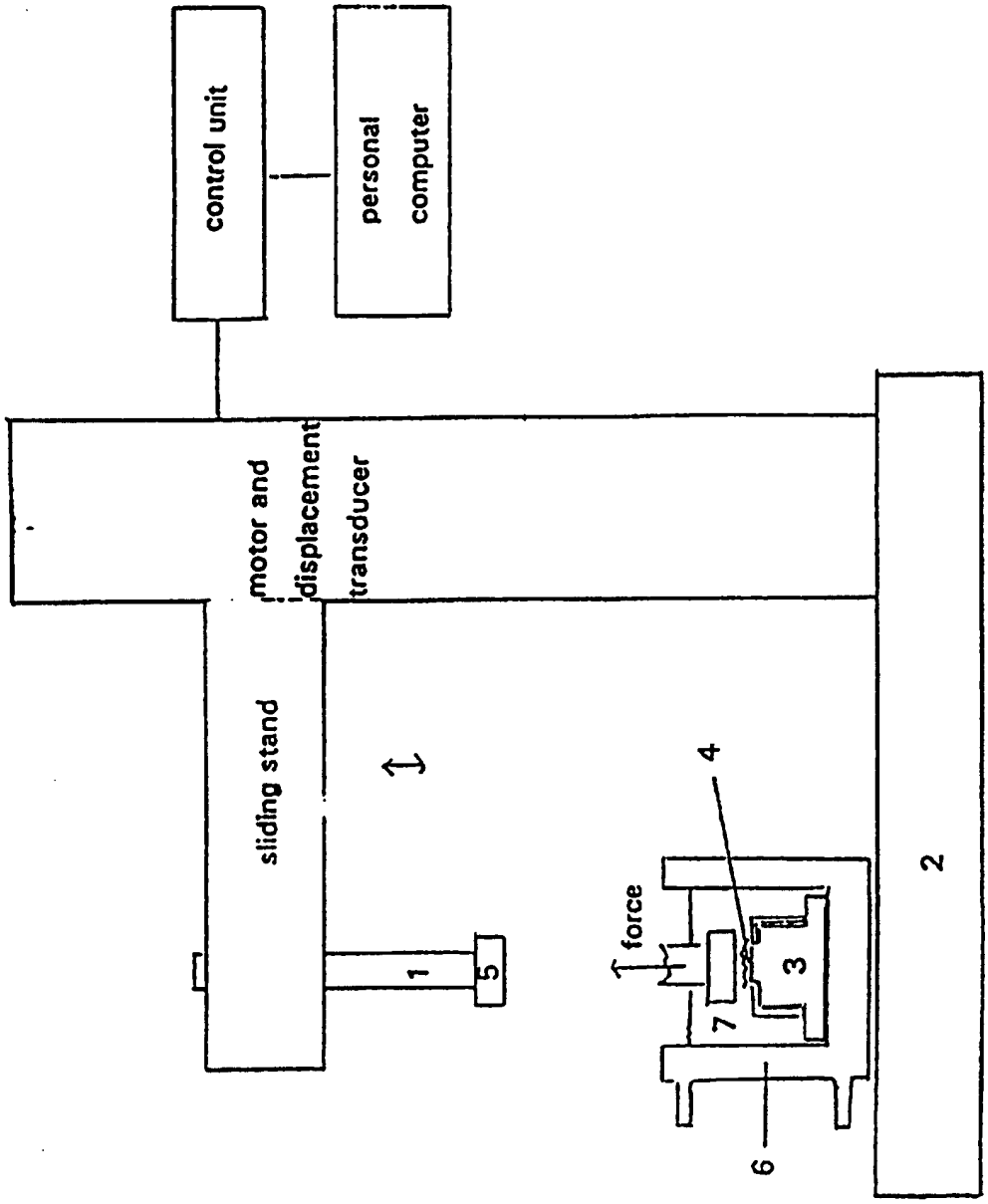
control unit
personal computer
motor and displacement transducer
sliding stand
force
1
2
3
4
5
6
7

Fig. 2*b*

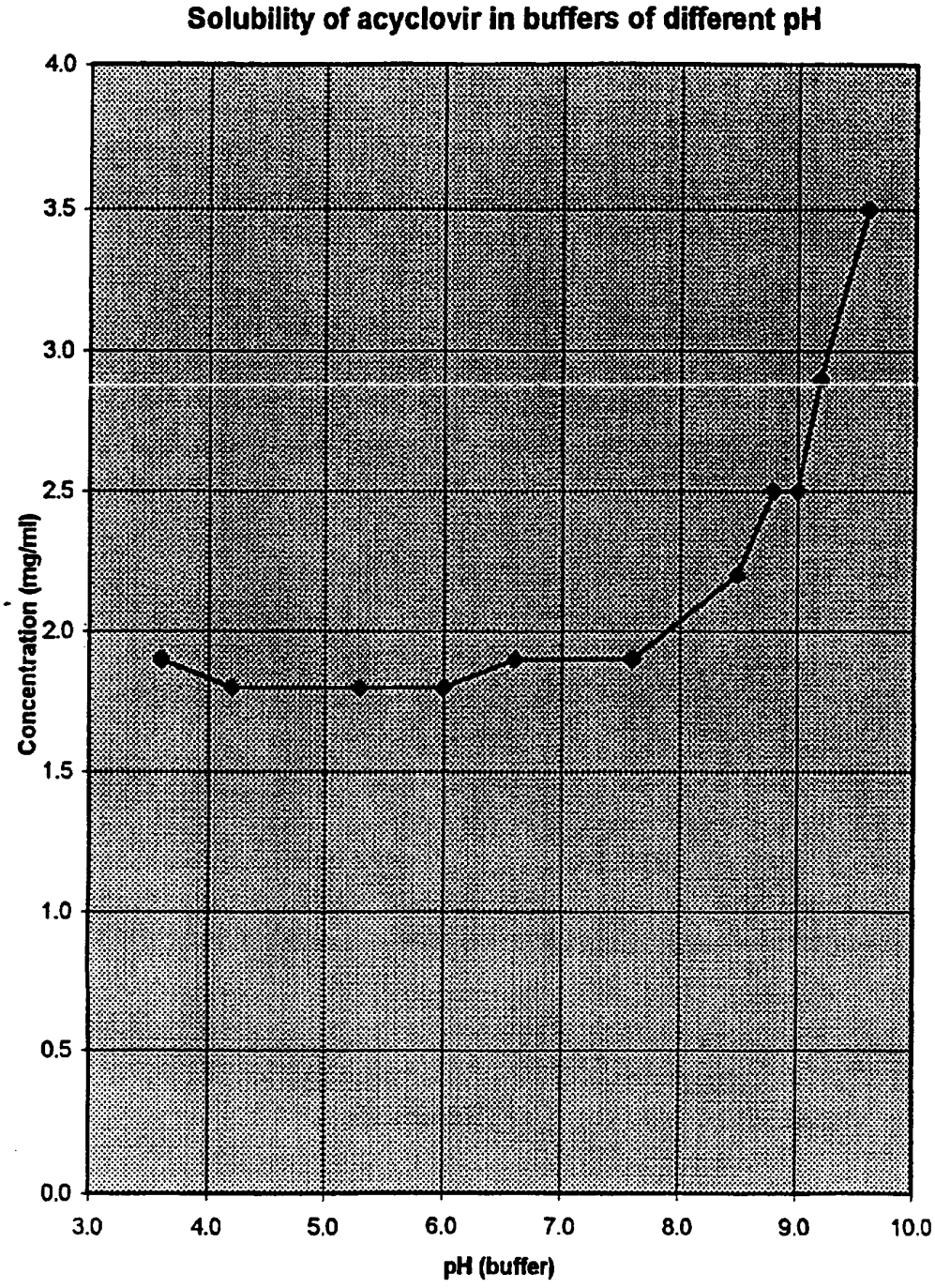
Solubility of acyclovir in buffers of different pH
Concentration (mg/ml)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
pH (buffer)

Fig. 3

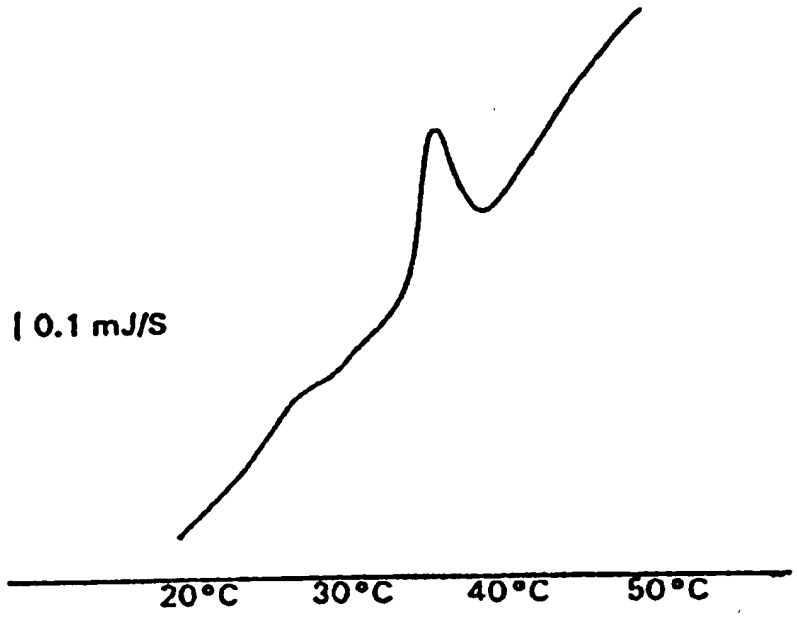

Fig. 4

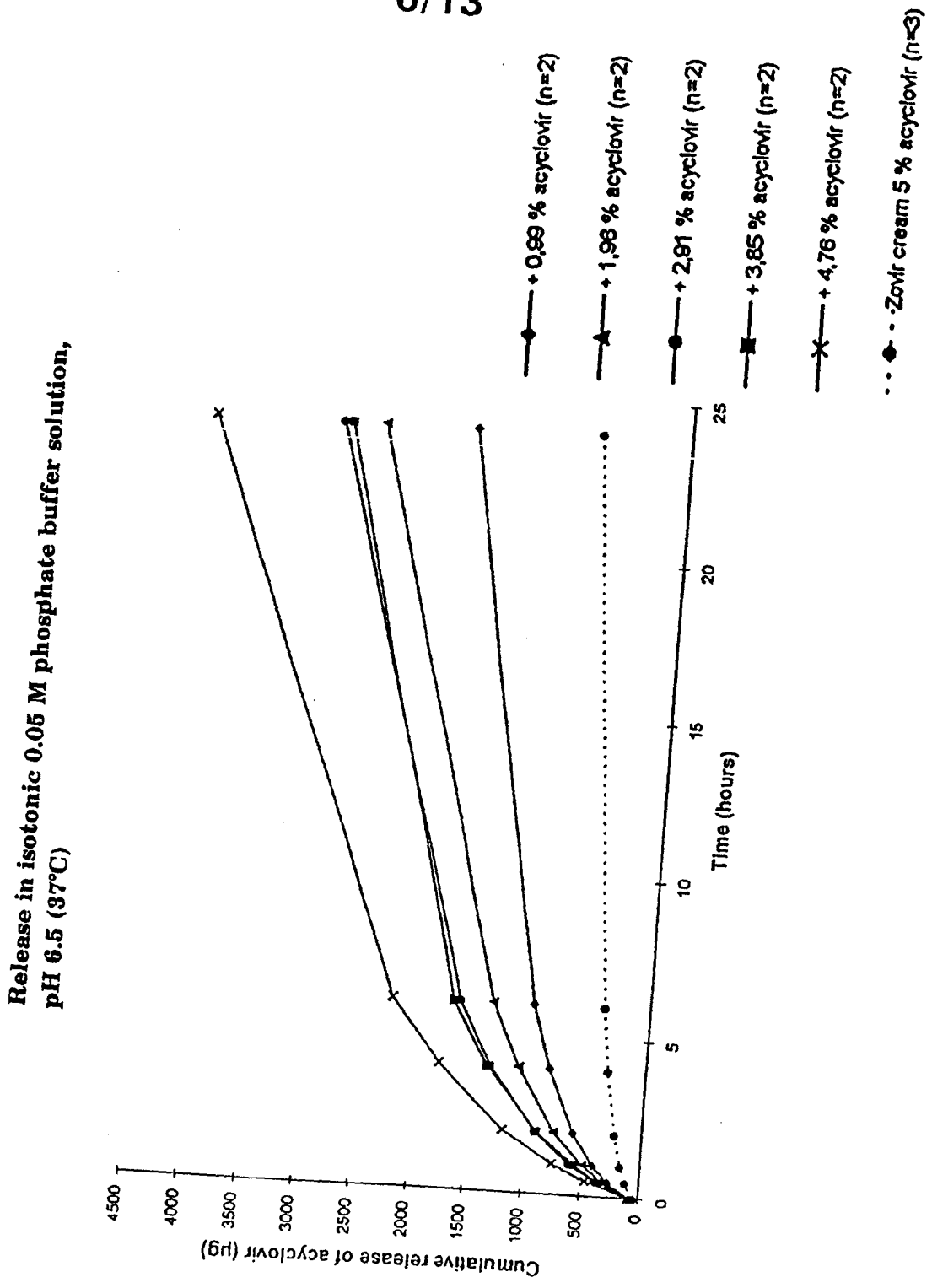
Release in isotonic 0.05 M phosphate buffer solution, pH 6.5 (37°C)
+ 0,99 % acyclovir (n=2)
+ 1,98 % acyclovir (n=2)
+ 2,91 % acyclovir (n=2)
+ 3,85 % acyclovir (n=2)
+ 4,76 % acyclovir (n=2)
Zovir cream 5 % acyclovir (n=3)
Cumulative release of acyclovir (µg)
Time (hours)
0
500
1000
1500
2000
2500
3000
3500
4000
4500
0
5
10
15
20
25

Fig. 5

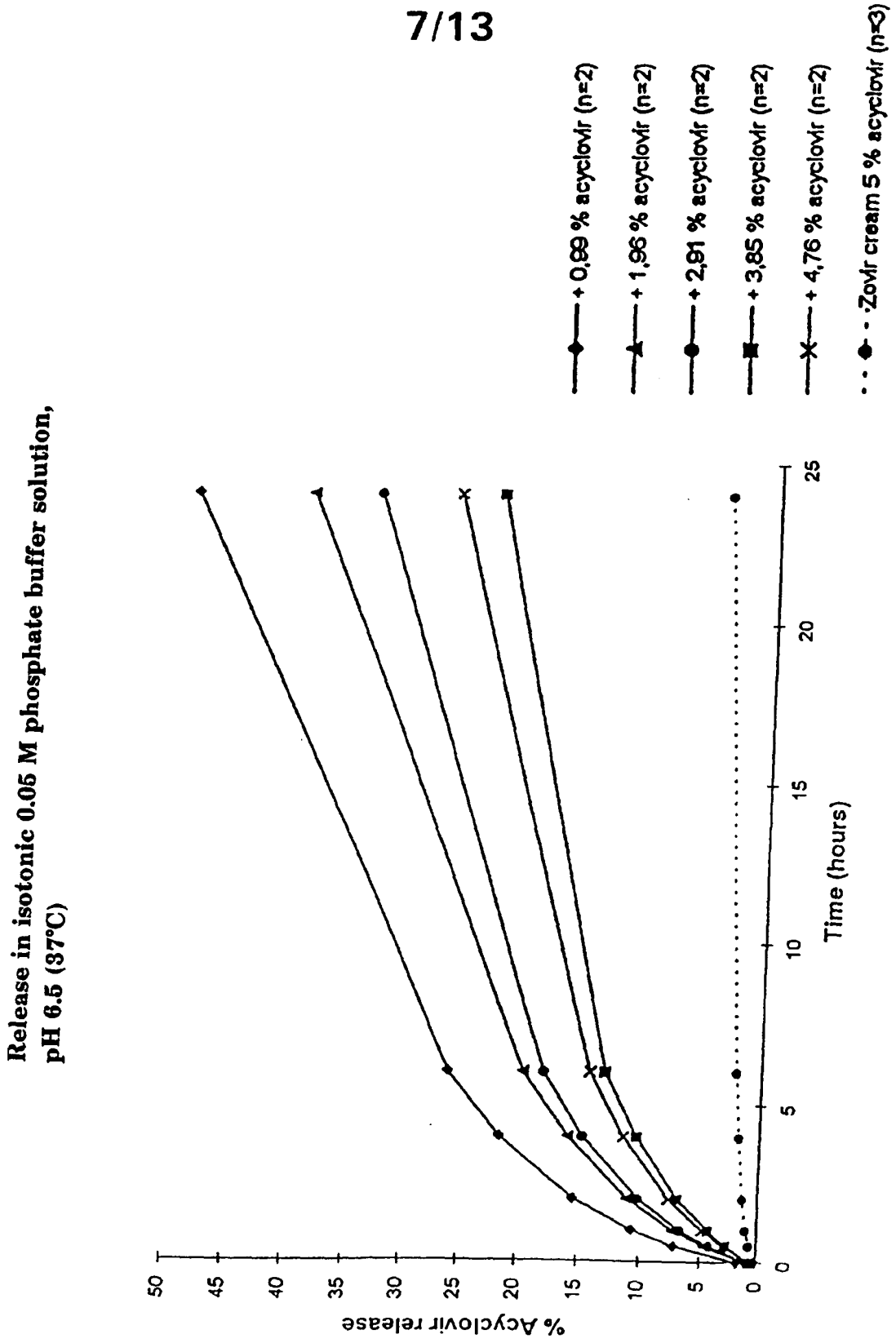
Release in isotonic 0.05 M phosphate buffer solution, pH 6.5 (37°C)
+ 0,89 % acyclovir (n=2)
+ 1,96 % acyclovir (n=2)
+ 2,91 % acyclovir (n=2)
+ 3,85 % acyclovir (n=2)
+ 4,76 % acyclovir (n=2)
- - -Zovir cream 5 % acyclovir (n=3)
% Acyclovir release
0
5
10
15
20
25
30
35
40
45
50
Time (hours)
0
5
10
15
20
25

Fig. 6

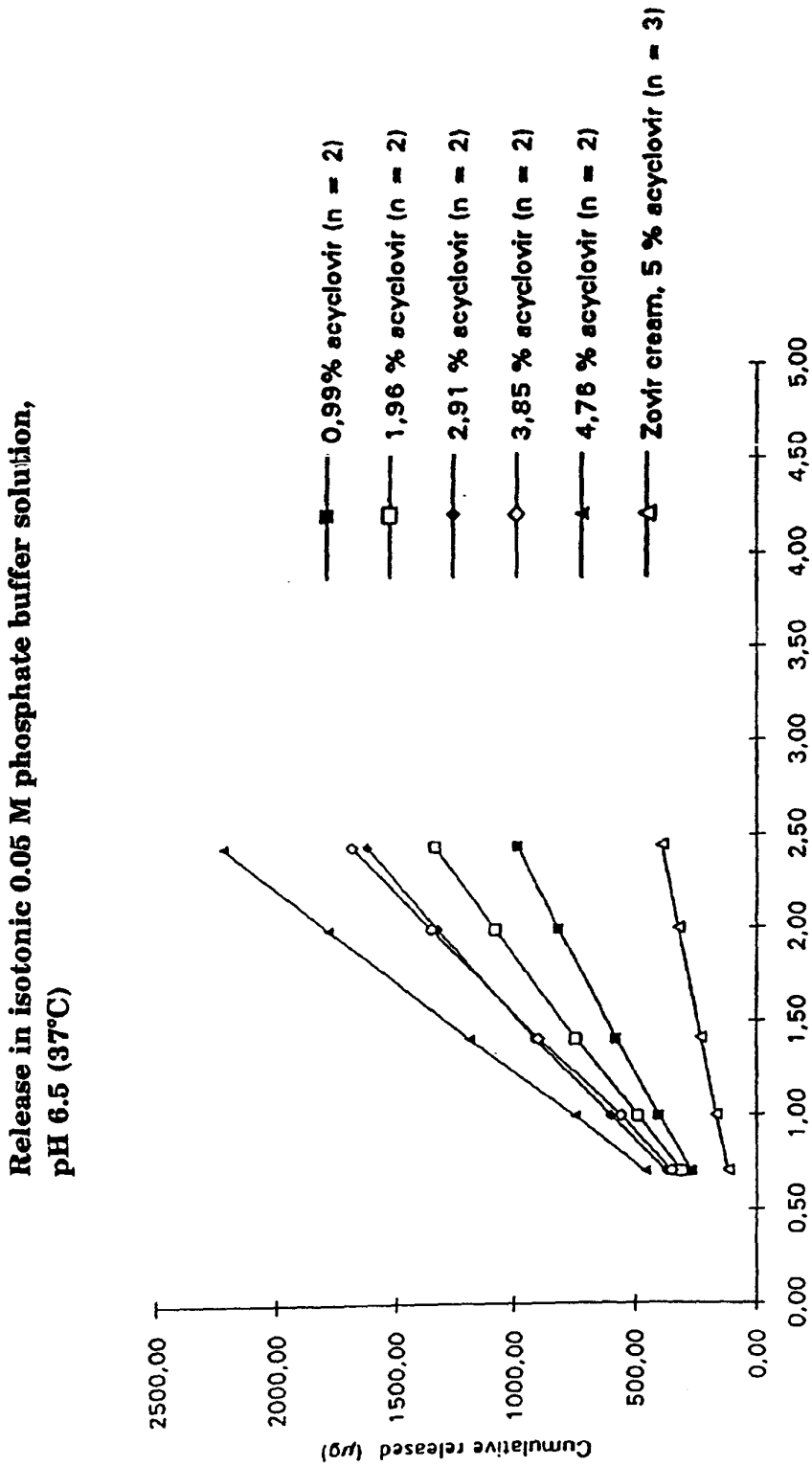
Release in isotonic 0.05 M phosphate buffer solution, pH 6.5 (37°C)
0,99% acyclovir (n = 2)
1,96 % acyclovir (n = 2)
2,91 % acyclovir (n = 2)
3,85 % acyclovir (n = 2)
4,78 % acyclovir (n = 2)
Zovir cream, 5 % acyclovir (n = 3)
Cumulative released (µg)
2500,00
2000,00
1500,00
1000,00
500,00
0,00
0,00
0,50
1,00
1,50
2,00
2,50
3,00
3,50
4,00
4,50
5,00
SQRT (t, (hours))

Fig. 7

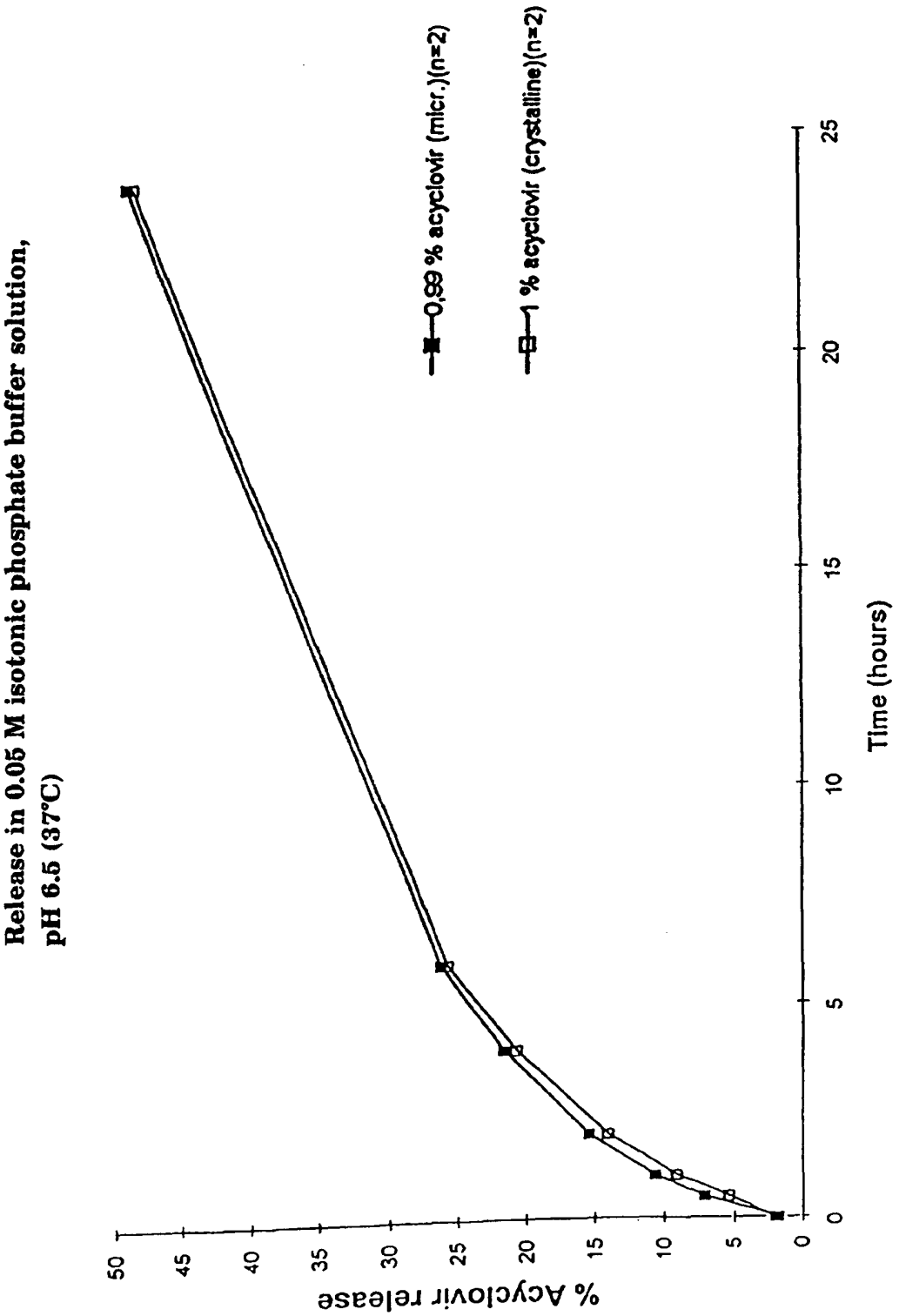
Release in 0.05 M isotonic phosphate buffer solution, pH 6.5 (37°C)
0,89 % acyclovir (micr.)(n=2)
1 % acyclovir (crystalline)(n=2)
% Acyclovir release
Time (hours)
0
5
10
15
20
25
30
35
40
45
50

Fig. 8

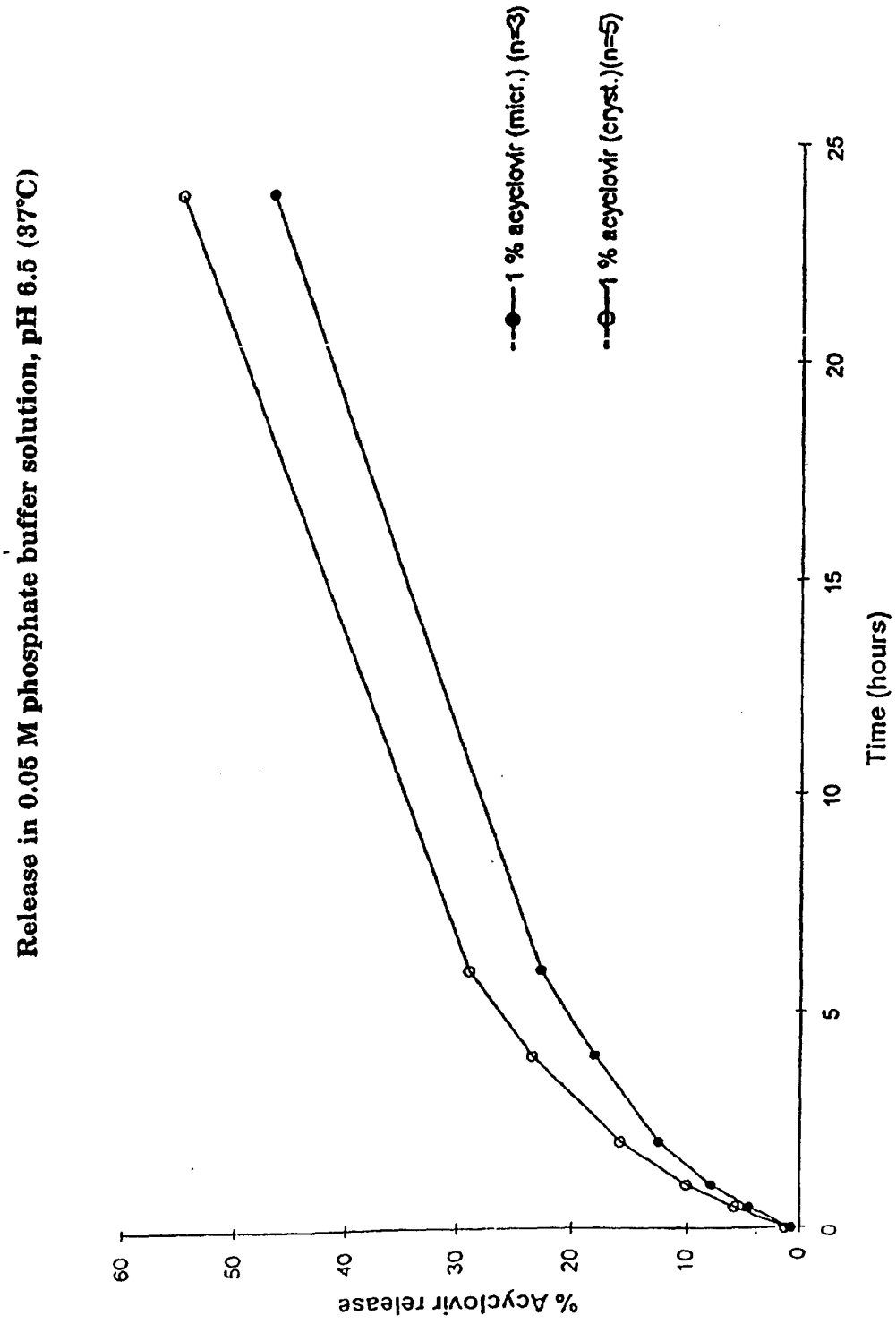
Release in 0.05 M phosphate buffer solution, pH 6.5 (37°C)
1 % acyclovir (micr.) (n=3)
1 % acyclovir (cryst.)(n=5)
% Acyclovir release
0
10
20
30
40
50
60
Time (hours)
0
5
10
15
20
25

Fig. 9

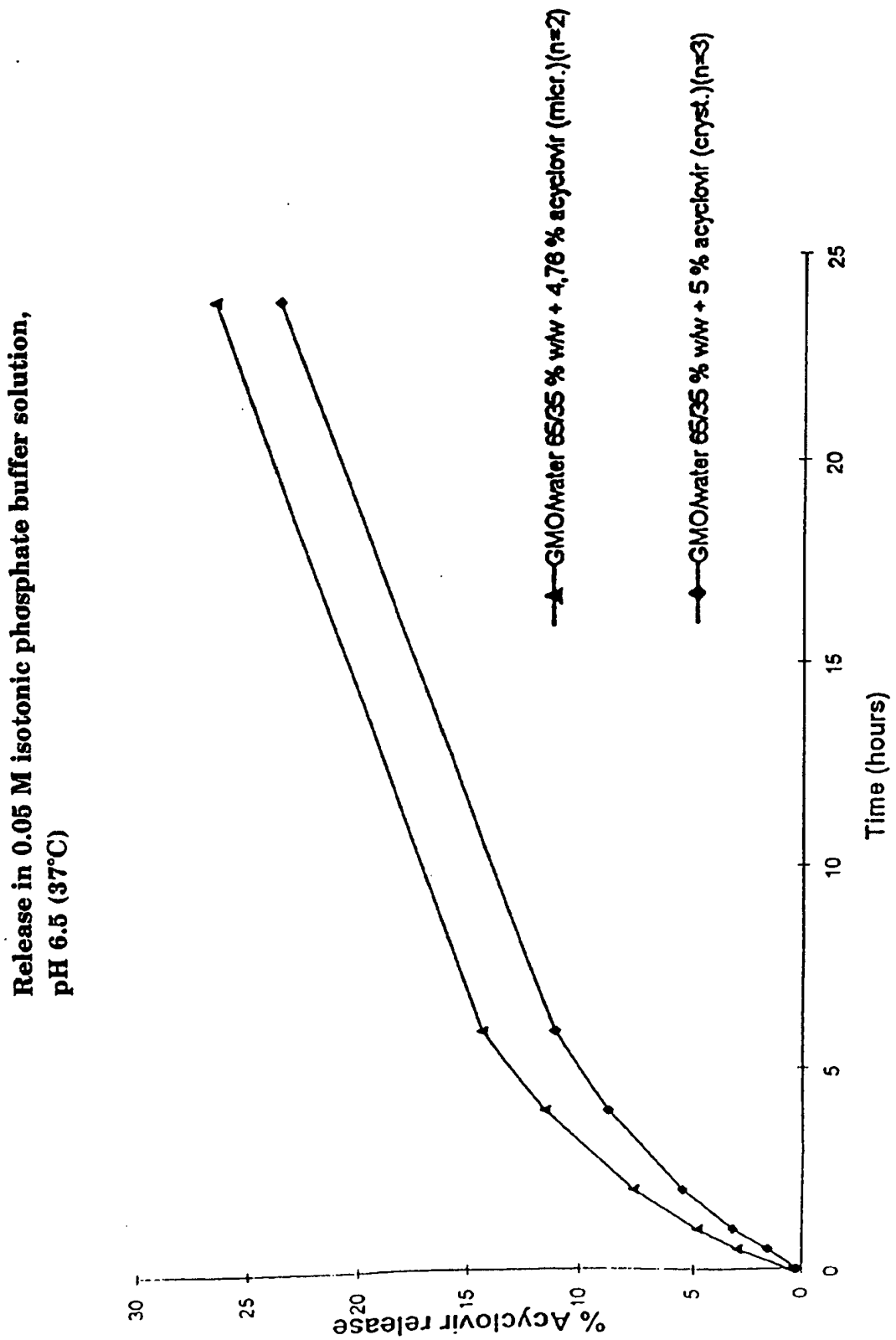
Release in 0.05 M isotonic phosphate buffer solution, pH 6.5 (37°C)
GMO/water 65/35 % w/w + 4,78 % acyclovir (micr.)(n=2)
GMO/water 65/35 % w/w + 5 % acyclovir (cryst.)(n=3)
% Acyclovir release
Time (hours)
0
5
10
15
20
25
30

Fig. 10

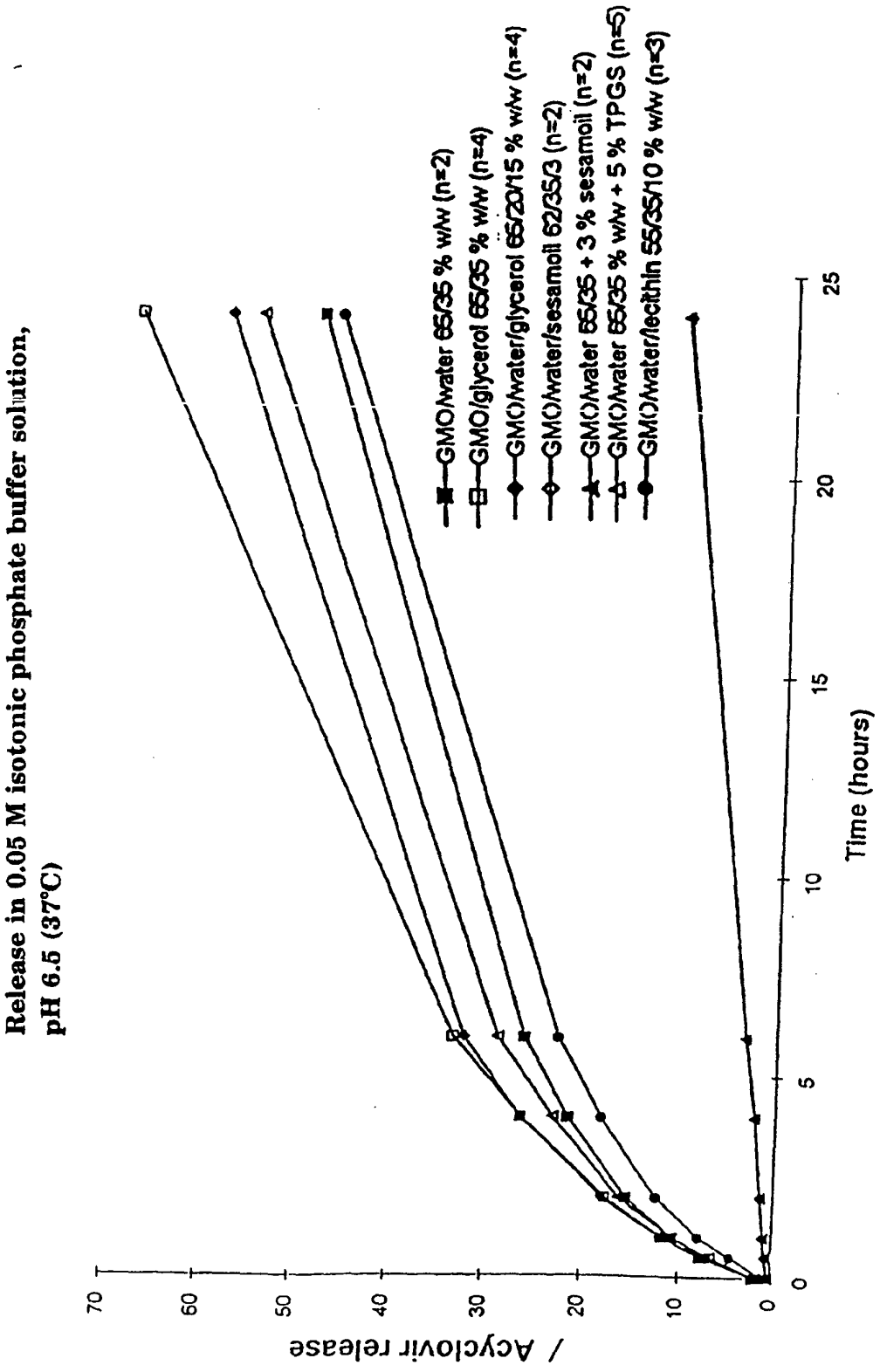
Release in 0.05 M isotonic phosphate buffer solution, pH 6.5 (37°C)
GMO/water 65/35 % w/w (n=2)
GMO/glycerol 65/35 % w/w (n=4)
GMO/water/glycerol 65/20/15 % w/w (n=4)
GMO/water/sesamoil 62/35/3 (n=2)
GMO/water 65/35 + 3 % sesamoil (n=2)
GMO/water 65/35 % w/w + 5 % TPGS (n=5)
GMO/water/lecithin 55/35/10 % w/w (n=3)
Acyclovir release / %
Time (hours)
0
10
20
30
40
50
60
70
0
5
10
15
20
25

Fig. 11

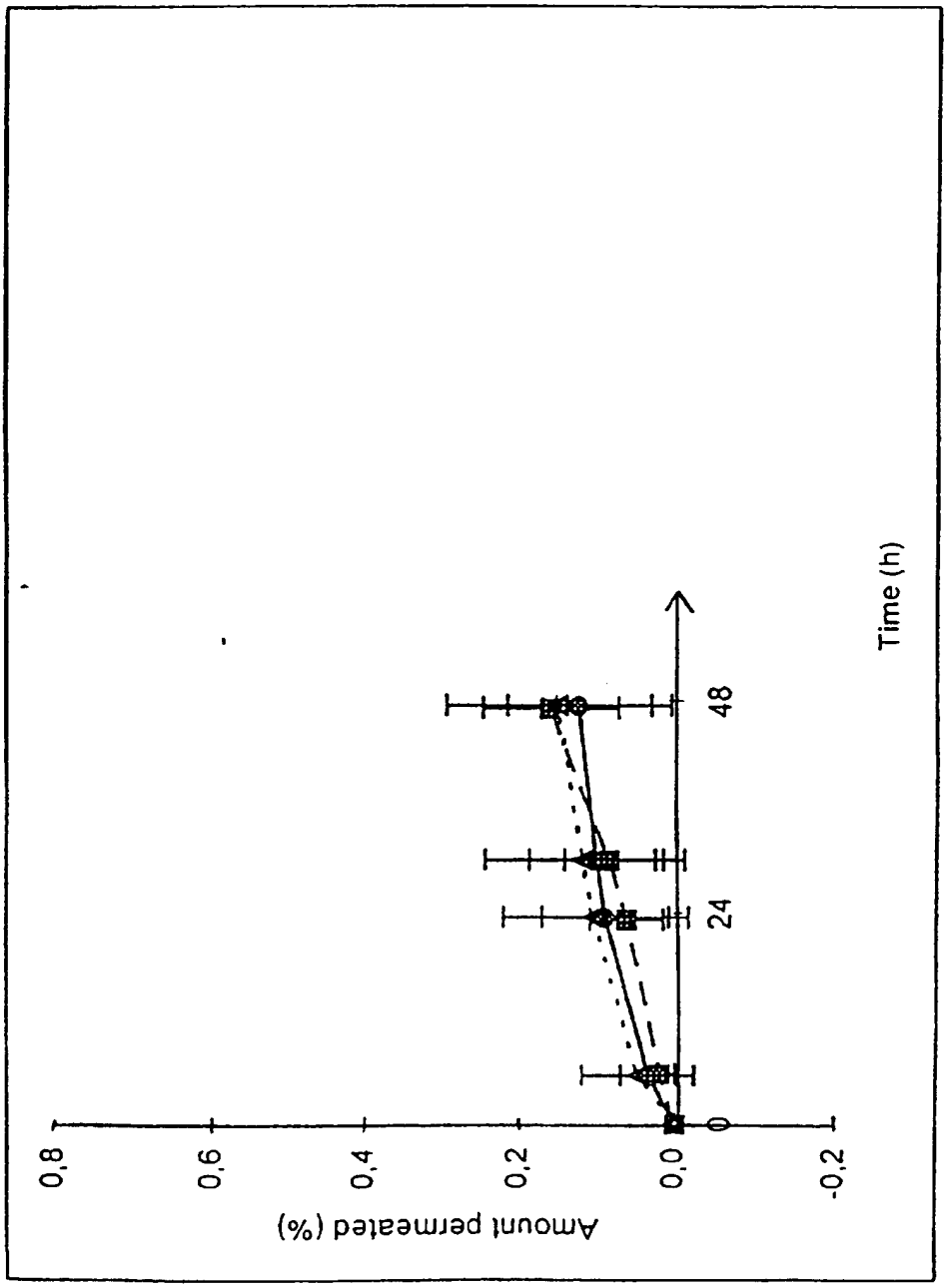

Fig. 1. Cumulative amount permeated in % of amount applied test substance.
■ = Zovir® cream, ● = GMO/water (5 % micronized acyclovir) and
▲ = GMO/water (5 % crystalline acyclovir).

Fig. 12

INTERNATIONAL SEARCH REPORT

Int onal Application No
PCT/DK 96/00437

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K47/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	WO 95 26715 A (A/S DUMEX) 12 October 1995 see the whole document see page 58, line 21 - line 23 *DOCUMENT CITED IN THE APPLICATION* ---	1-91
A	EP 0 299 937 A (LARSSON) 18 January 1989 see the whole document see column 3, line 47 - line 50 ---	1-91
A	LIPID TECHNOLOGY, vol. 2, no. 2, April 1990, pages 42-45, XP002002714 S. ENGSTRÖM: "DRUG DELIVERY FROM CUBIC AND OTHER LIPID-WATER PHASES" see the whole document --- -/--	1-91

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 January 1997	31.01.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Benz, K

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/DK 96/00437

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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